"FORMULATION & EVALUATION OF MICROEMULSION IN SITU GEL OF NATAMYCIN: OCULAR DRUG DELIVERY"

A Thesis Submitted to

NIRMA UNIVERSITY

In Partial Fulfillment for the Award of the Degree of

MASTER OF PHARMACY

IN

PHARMACEUTICAL TECHNOLOGY AND BIOPHARMACEUTICS

BY

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ACKNOWLEDGEMENT

The joyness, satisfaction and euphoria that come along with successful completion of any work would be incomplete unless we mention the name of those people who made it possible whose constant guidance and encouragement served as a beam of light and crowed out efforts.

First of all, I am thankful to ***Almighty (The Supreme Soul)*** *for always being with me and blessing me with good family, friends, teachers and well wishers and extend his helping hands to complete this project successfully.*

No research is ever the outcome of a single individual's efforts. This work is no exception. Some of the personalities known and unknown, which contributed to this project made it possible for me to present it in this format. I am extremely grateful to all of them.

I am highly thankful to ***Dr. Manjunath Ghate*** *(Director, Institute of Pharmacy, Nirma University, Ahmedabad) for providing all necessary help and facility for my work and also for his constant support and encouragement.*

I express my warmest gratitude to my guide ***Dr. Mayur M Patel,*** *(Asst. Professor, Dept of Pharmaceutics, Institute of Pharmacy, Nirma University) for his valuable guidance, keen interest, perennial inspiration and everlasting encouragement. It is with affection and reverence that I acknowledge my indebtness to his for outstanding dedication, often far beyond the call of duty. I am extremely indebted to his for motivational inspiration, kind expertise during the writing up of my thesis and the scientific attitude they have nurtured in me which will definitely stand in all my future endeavours.*

I am thankful to ***Dr. Jigar Shah****, Assistant Professor, Dept of Pharmaceutics, Institute of Pharmacy, Nirma University for his timely suggestions and total understanding.*

I am thankful to ***Dr. Tejal A Mehta****, Professor, Head, Dept of Pharmaceutics, Institute of Pharmacy, Nirma University for her unending encouragement, friendly nature, timely suggestions and total understanding.*

I am extremely grateful to ***Dr. Shital Butani, Dr. Renuka D Mishra, Mr. Dhaivat Parikh,*** *Dept of Pharmaceutics, Institute of Pharmacy, Nirma University for their continues encouragement and everlasting support throughout the course of this dissertation work.*

*I express my special thanks to Dr****. Tejal A Mehta****, Professor, Head of Department, Pharmaceutics and Pharmaceutical Technology, Institute of Pharmacy, Nirma University,* ***Dr. Anuradha K. Gajjar****, Professor, Department of Medicinal Chemistry, Institute of Pharmacy, Nirma University,* ***Dr. Priti J Mehta,*** *Associate Professor, Head of Department, Pharmaceutical Analysis, Institute of Pharmacy, Nirma University for their constant moral support and kind cooperation.*

I would like to thank specially to ***Dr Bhoomika M Patel, Dr. Snehal D Patel****, Assistant Professor, Dept of Pharmacology, Institute of Pharmacy, Nirma University for their selfless support, co-operation and valuable suggestion.*

I owe special words of thank to ***Shailesh Bhai, Mukesh Patel and Shilpaben*** *for their precious help.*

Words are an inadequate medium to express my feelings and deep sense of gratitude to my beloved ***parents*** *who have always given me moral support & encouragement. Their prayers to God have made me achieve this milestone of my carrier. Their support and encouragement with their unending cheering have led to the successful completion of my research work.*

I am heartily thankful to all my classmates and friends especially ***Chandni, Tejas, Pankaj, Arun, Amisha, Rupak, Divij, Rakesh, Girish and Jayesh*** *for giving me constant support and help during my dissertation work. Words do not suffice to express my sincere and humble thanks to all my very special friends. I would like to thank my juniors* ***Amit, Chintan, Dhruvi, Dishant, Kashyap, Keyur, Mahima, Manali, Mahulik, Nirav, Rashmi, Ronak, Sagar, Tora, Utkarsh, Zeal, Rohit, Raghu, Subash.***

I would like to thank specially to Ph.D students ***Prerak sir, Som sir, Omkar sir and Jignesh Sir*** *for their selfless support, co-operation and valuable suggestion.*

*I am also thankful to my friends **Kewal, Margi, Yesha** for providing me selfless support.*

Last, but not the least, I express my gratitude and apologize to anybody whose contributions, I could not mention in this page.

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INDEX

Sr. No.	Title		Page No.
1.	**Aim of present investigation**		1
2.	**Introduction**		3
	2.1	Introduction to eye	3
	2.2	Introduction of microemulsion	9
	2.3	In situ gelling system	14
	2.4	Introduction of drug	16
	2.5	Introduction of excipient	20
3.	**Literature review**		33
4.	**Material and method**		
	4.1	List of materials	42
	4.2	Instrument used	43
	4.3	Identification of natamycin	44
	4.4	Estimation of natamycin	44
	4.5	Solubility studies and selection of surfactant and oil component	45
	4.6	Evaluation of microemulsion	45
	4.8	In situ gelling system:	48
	4.9	Formulation of microemulsion in situ gelling system	50
5	**Experimental Work**		
	5.1	Identification of natamycin	52
	5.2	Estimation of natamycin	54
	5.3	Selection of oil and surfactant by solubility	57
	5.4	Construction of pseudo-ternary phase diagrams:	59
	5.5	Microemulsion preparation	73
	5.6	In situ gel	86
	5.7	Screening of bioadhesive polymer	88
6.	**Summary**		92
7.	**Future prospective**		94
8.	**References**		95

LIST OF TABLES

2.1	Stimuli sensitivity of hydrogels	15
2.2	Specification of natamycin	18
2.3	Uses of carbomer	22
2.4	Typical viscosity values for 2% (w/v) aqueous solution of methocel	25
2.5	Uses of polysorbate	28
5.1	Compare melting point of Natamycin	52
5.2	Standard curve of Natamycin in Methanol	54
5.3	Regression Analysis for Standard curve of Natamycin in Methanol	55
5.4	Standard Curve of Natamycin in STF	55
5.5	Regression Analysis for Standard Curve of Natamycin in STF	56
5.6	Solubility of Natamycin in various Oil	57
5.7	Solubility of natamycin in Various Surfactant:	58
5.8	Component of mixture surfactant : cosurfactant (1:1) and IPM that show in the formation of microemulsion	60
5.9	Component of mixture surfactant : cosurfactant (2:1) and IPM that show in the formation of microemulsion	61
5.10	Component of mixture surfactant : cosurfactant (1:2) and IPM that show in the formation of microemulsion	62
5.11	Component of mixture surfactant : cosurfactant (1:3) and IPM that show in the formation of microemulsion	63
5.12	Component of mixture surfactant : cosurfactant (1:4) and IPM that show in the formation of microemulsion	64
5.13	Component of mixture surfactant : cosurfactant (1:5) and IPM that show in the formation of microemulsion	65
5.14	Component of mixture surfactant : cosurfactant (1:6) and IPM that show in the formation of microemulsion	66
5.15	Component of mixture surfactant : cosurfactant (1:7) and IPM that show in the formation of microemulsion	67
5.16	Compositon of Natamycin (1%) loaded Microemulsion	73
5.17	Result of of natamycin (1%) loaded microemulsion	82
5.18	Drug Content	84
5.19	In vitro permeation data of eye drop and microemulsion Batch A	85
5.20	Preparation of carbopol 940 In-situ ophthalmic gel (Optimization of carbopol 940 concentartion)	86
5.21	Preparation of carbopol 940 In-situ ophthalmic gel (Carbopol 940 +Methyl cellulose)	88
5.22	Preparation of carbopol 940 In-situ ophthalmic gel (Carbopol 940 + HPMC K4M)	89

LIST OF FIGURES

Sr. No.	Title	Page No.
2.1	Structure of Eye-Ball	3
2.2	Nasolachrymal Drainage	5
2.3	Mechanism of Ocular Drug Absorption	6
2.4	Ternary Phase Diagram	12
4.1	Diffusion Assembly Set up	47
5.1	UV spectra of Natamycin in Methanol + 0.1 % Glacial acetic acid	53
5.2	Standard curve of Natamycin in Methanol	54
5.3	Standard Curve of Natamycin in STF	56
5.4	Solubility of natamycin in various Oil	57
5.5	Solubility of natamycin in various Surfactant	58
5.6	Ternary phase diagram of 1:1 Surafactant : cosurfactant and Oil	60
5.7	Ternary phase diagram of 2:1 Surafactant : cosurfactant and Oil	61
5.8	Ternary phase diagram of 1:2 Surafactant : cosurfactant and Oil	62
5.9	Ternary phase diagram of 1:3 Surafactant : cosurfactant and Oil	63
5.10	Ternary phase diagram of 1:4 Surafactant : cosurfactant and Oil	64
5.11	Ternary phase diagram of 1:5 Surafactant : cosurfactant and Oil	65
5.12	Ternary phase diagram of 1:6 Surafactant : cosurfactant and Oil	66
5.13	Ternary phase diagram of 1:7 Surafactant : cosurfactant and Oil	67
5.14	Percentage microemulsion existence area	68
5.15	Ternary Phase Digram all Point where microemulsion existence	72
5.16	Comparison of particle size of various microemulsion batches	74
5.17	Comparison of solubility of various microemulsion batches	74
5.18	Comparison of in vitro permeation study (%) of various microemulsion Batches	84
5.19	Rheological profile of in situ gelling system	90
5.20	Comparision of in vitro release of of in situ gelling system	91

LIST OF ABBREVIATIONS

Short name	Abbreviation
IP	India Pharmacopoeia
BP	British Pharmacopoeia
USP	United States Pharmacopeia
PhEur	European Pharmacopoeia
USPNF	United States Pharmacopoeia National Formulary
UV	Ultra Violet
NaCl	Sodium chloride
KBr	Potassium Bromide
°C	Degree centigrade
Conc.	Concentration
CPR	Cumulative Percentage Release
µg	Microgram
SD	Standard Deviation
Avg.	Average
Hr	Hour
Min	Minute
STF	Simulated tear fluid
BKC	Benzalkonium chloride

Chapter 1
Aim

AIM OF PRESENT WORK

Natamycin also known as pimaricin, is a naturally occurring antifungal agent produced during fermentation by the bacterium Streptomyces natalensis, commonly found in soil. Natamycin is classified as a macrolide polyene antifungal and, as a drug, is used to treat fungal keratitis. It is especially effective against Aspergillus and Fusarium corneal infections.

- Natamycin is a polyene antibiotic, poorly absorbed from the gastrointestinal tract, whose half life (t½) of 2-3 hr and praciticaly insoluble (30-50 mg/L) in water.
- It is presently available in market in suspension forms.

Drawback of Suspension :

- low stability
- low permeability through coneal membrane

To overcome problem related suspension need develop microemulsion system of Natamycin.

Microemulsion have high solubility capacity, thermodynamically stable solution ,low particle size so that increase permeability through corneal membrane.

Frequency of dosage :

Natamycin has low half life 2-3 hr so its frequency of dosage is very high

Frequency of dosage of Marketed Natamycin suspension (Natamet) :

Instill 1 drop in conjuctival sac every 1-2 hr, reduce drop 6-8 times a day after 3-4 day, Duration of treatment is 2-3 week

Dosing frequency of marketed formulation is very high which show that formulate dosage form to reduce dose frequency for better patient compliance.

Conventional ophthalmic formulation like eye drops exhibits many drawbacks like rapid pre-corneal drainage and poor bioavailability. They cannot provide sufficient concentration of drug in posterior tissues [1]. Novel formulations like liposomes, niosomes and other microparticulate systems are commonly employed for invasive

delivery and they are very expensive [2]. This indicates strong need to formulate a dosage form, which is economic and efficient to overcome the drawbacks of existing ophthalmic formulations.

The in situ drug delivery system and colloidal formulation like microemulsion has potential to use in ocular delivery. Microemulsion provides better permeation of drug through the membrane and provides improved bioavailability [3, 4]. The in situ drug delivery system decreases pre-corneal drainage, increase the contact time of formulation with eye and prolong the release in ocular tissues. Again, in situ gelling system has advantage of delivering accurate and reproducible quantities, in contrast to already gelled formulations. To exploit the benefits of these two dosage forms, microemulsion based in situ gelling system was developed as a new vehicle for ophthalmic drug delivery [5]. The essential idea is to encapsulate the drug in droplets that form a microemulsion, and then disperse the drug-loaded droplets in a polymer solution that gels upon triggering by the pH present in the tear fluid.

Chapter 2
Introduction

2.1 INTRODUCTION TO EYE [6-8]

2.1.1 ANATOMY AND FUNCTION OF THE EYE

The eye is a spherical structure with a wall consisting of three layers; the outer sclera, the middle choroid layer, Ciliary body and iris and the inner nervous tissue layer retina. The sclera is tough fibrous coating that protects the inner layers. It is white except for the transparent area at the front, the cornea which allow light to enter the eye. The choroid layer, situated inside the sclera, contains many blood vessels and is modified at the front of the eye as pigmented iris. The iris is the coloured part of the eye (in shades of blue, green, brown, hazel, or grey).

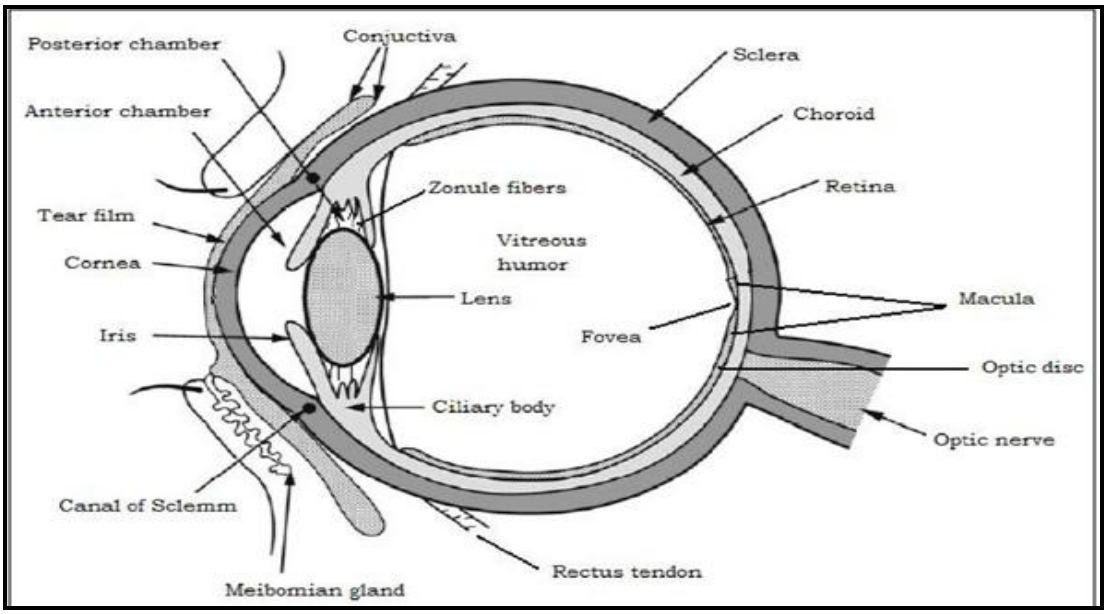


Figure 2.1: Structure of Eye-Ball

2.1.2 THE STRUCTURE OF THE CORNEA

The cornea is a strong clear transparent bulge located at the front of the eye that conveys images to the back of the eyes. The front surface of the adult cornea has a radius of approximately 8mm that covers about one-sixth of the total surface of the eye ball. It is a vascular tissue to which nutrient and oxygen are supplied via bathing with lachrymal fluid and aqueous humour as well as from blood vessels that lines the junction between the cornea and sclera (fig.2.1).

The cornea is the main pathway permeation of drug into the eye. It is composed of five layers: epithelium, Bowman's layer, stroma, Descemet's membrane and endothelium. The epithelium consists of 5 to 6 layers of cells. The corneal thickness is 0.5–0.7 mm and it is thicker in the central region. The corneal epithelium is the main barrier of drug absorption into the eye in Comparison to many other epithelial tissues (intestinal, nasal, bronchial, and tracheal) corneal epithelium is relatively impermeable. The epithelium is squamous stratified, consisting of 5-6 layer of cells with a total thickness around 50-100 μm and turnover of about one cell layer per day. The basal cells are packed closely together with a tight junction, to forming not only an effective barrier to most microorganisms, but also for drug absorption. Drugs penetrate across the corneal epithelium via the transcellular or paracellular pathway. Lipophilic drugs prefer the transcellular route and hydrophilic drugs penetrate primarily through the paracellular pathway which involves passive or altered diffusion through intercellular spaces. For most topically applied drugs, passive diffusion along their concentration gradient, either transcellularly or paracellularly, is the main permeation mechanism across the cornea.

2.1.3 CONJUNCTIVA

The conjunctiva is involved in the formation and maintenance of the precorneal tear film and in the protection of the eye. The conjunctiva is a thin transparent membrane, which lines the inner surface of the eyelids and is reflected onto the globe. The membrane is vascular and moistened by the tear film. The conjunctiva is composed of an epithelium, a highly vascularised substantia propria, and a submucosa or episclera. The bulbar epithelium consists of 5 to 7 cell layers. The structure resembles a palisade and not a pavement when compared to the corneal epithelium. At the surface, epithelial cells are connected by tight junctions, which render the conjunctiva relatively impermeable. The conjunctival tissue is permeable to molecules up to 20,000 Da, whereas the cornea is impermeable to molecules larger than 5000 Da. The human conjunctiva is between 2 and 30 times more permeable to drugs than the cornea and it has been proposed that loss by this route is a major path for drug clearance. There are 1.5 million globlet cell present in the conjunctiva with the highest density is in the Inferonasal quadrant (10 goblet cells/mm2). The highest

density found in the children and adults varying with age depended among the intersujects variability. A significant increase in the number of goblet cells was reported in the case of vernal conjunctivitis and atopic kerato conjunctivitis but a great variation in goblet cell density results only in a small difference in tear mucin concentration.

2.1.4 NASOLACHRYMAL DRAINAGE SYSTEM

Nasolachrymal drainage system consists of three parts; the secretory system, the distributive system and the excretory system. The secretory portion is composed of the lacrimal gland that secreted tears are spread over the ocular surface by the eyelids during blinking. The secretory system is stimulated by blinking and temperature change due to the tear evaporation and reflux secretors that have an efferent parasympathetic nerve supply and secrete in response to physical and emotional stimulation e.g. crying. The distributive system consists of the eyelids and the tear meniscus around the lid edges of the open eye, which spread tears over the ocular surface by blinking, thus preventing dry areas from developing. The excretory part of the Nasolachrymal drainage system consists of the lachrymal puncta, the superior, inferior and common canaliculi; the lachrymal sac, and the nasochrymal duct. In humans, the two puncta are the openings of the lachrymal canaliculi and are situated on an elevated area known as the lachrymal papilla. It is thought that tears are largely absorbed by the mucous membrane that lines the ducts and the lachrymal sac; only a small amount reaches the nasal passage.

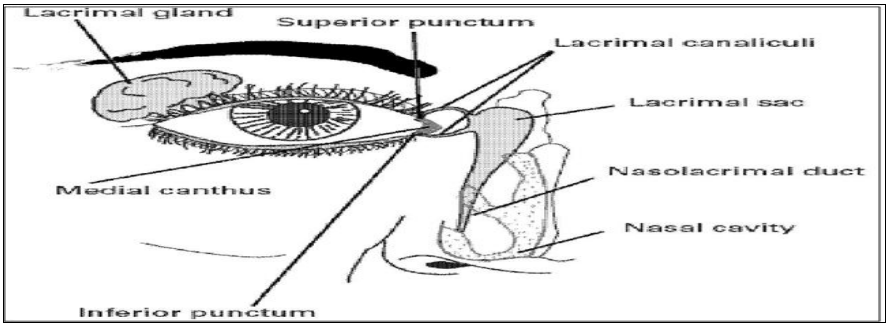


Figure 2.2: Nasolachrymal Drainage

2.1.5 MECHANISM OF OCULAR DRUG ABSORPTION

Drugs administered by instillation must penetrate the eye and do so primarily through the cornea followed by the non-corneal routes. These non-corneal routes involve drug diffusion across the conjunctiva and sclera and appear to be particularly important for drugs that are poorly absorbed across the cornea.

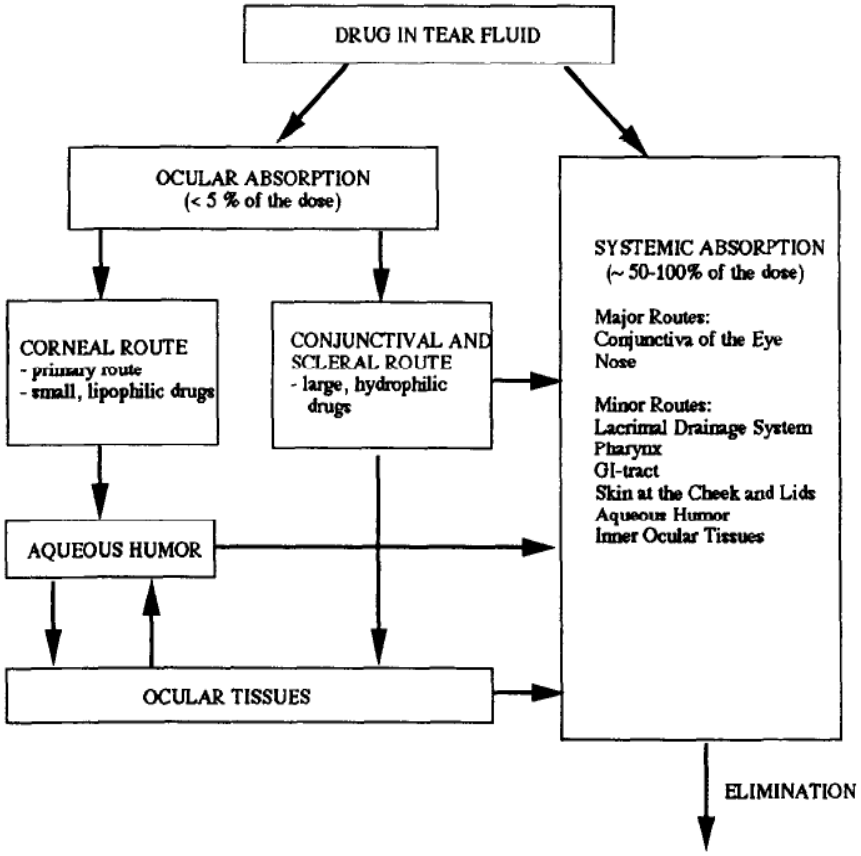


Figure: 2.3 Mechanism of Ocular Drug Absorption

2.1.6 BARRIERS FOR OCULAR DELIVERY

2.1.6.1 Drug loss from the ocular surface

After instillation, the flow of lacrimal fluid removes instilled compounds from the surface of the eye. Even though the lacrimal turnover rate is only about 1 µl/min the excess volume of the instilled fluid is flown to the nasolacrimal duct rapidly in a couple of minutes. Another source of non-productive drug removal is its systemic absorption instead of ocular absorption. Systemic absorption may take place either

directly from the conjunctival sac via local blood capillaries or after the solution flow to the nasal cavity.

2.1.6.2 Lacrimal fluid-eye barriers

Corneal epithelium limits drug absorption from the lacrimal fluid into the eye. The corneal epithelial cells form tight junctions that limit the paracellular drug permeation. Therefore, lipophilic drugs have typically at least an order of magnitude higher permeability in the cornea than the hydrophilic drugs. In general, the conjunctiva is leakier epithelium than the cornea and its surface area is also nearly 20 times greater than that of the cornea.

2.1.6.3 Blood-ocular barriers

The eye is protected from the xenobiotics in the blood stream by blood-ocular barriers. These barriers have two parts: blood-aqueous barrier and blood-retina barrier. The anterior blood-eye barrier is composed of the endothelial cells in the uvea (The middle layer of the eye beneath the the sclera. It consists of the iris, ciliary body, and choroid). This barrier prevents the access of plasma albumin into the aqueous humor, and also limits the access of hydrophilic drugs from plasma into the aqueous humor. The posterior barrier between blood stream and eye is comprised of retinal pigment epithelium (RPE) and the tight walls of retinal capillaries. Unlike retinal capillaries the vasculature of the choroid has extensive blood flow and leaky walls. Drugs easily gain access to the choroidal extravascular space, but thereafter distribution into the retina is limited by the RPE and retinal endothelia.

2.1.7 CONJUNCTIVITIS [9, 10]

2.1.7.1 DEFINITION

Conjuctivitis is an inflammation or redness of the lining of the white part of the eye and the underside of the eyelid (conjunctiva) that can be caused by infection, allergic reaction, or physical agents like infrared or ultraviolet light.

2.1.7.2 TYPE OF CONJUCTIVIS

- Allergic conjunctivitis
- Bacterial conjunctivitis
- Chemical conjunctivitis

ALLERGIC CONJUNCTIVITIS

Allergic conjunctivitis is inflammation of the conjunctiva (the membrane covering the white part of the eye) due to allergy. Although allergens differ between patients, the most common cause ishay fever. Symptoms consist of redness (mainly due to vasodilation of the peripheral small blood vessels), oedema of the conjunctiva, itching and (production of tears). If this is combined with rhinitis, the condition is termed allergic rhino conjunctivitis,

The conjunctiva is a thin membrane that covers the eye. When an allergen irritates the conjunctiva, common symptoms that occur in the eye include: ocular itching, eyelid swelling, tearing, photophobia, watery discharge, and foreign body sensation (with pain**).**

BACTERIAL CONJUNCTIVITIS

Bacterial conjunctivitis due to common pyogenic (pus-producing) bacteria causes marked grittiness/irritation and a stringy, opaque, greyish or yellowish mucopurulent discharge that may cause the lids to stick together, especially after slee Bacterial conjunctivitis due to common pyogenic (pus-producing) bacteria causes marked grittiness/irritation and a stringy, opaque, greyish or yellowish mucopurulent discharge that may cause the lids to stick together, especially after sleep.

CHEMICAL CONJUNCTIVITIS

Chemical eye injury is due to either an acidic or alkali substance getting in the eye. Alkalis are typically worse than acidic burns.[5] Mild burns will produce conjunctivitis while more severe burns may cause the cornea to turn white.[5] Litmus paper is an easy way to rule out the diagnosis by verifying that the pH is within the normal range of 7.0—7.2. Large volumes of irrigation is the treatment of choice and

should continue until the pH is 6—8. Local anaesthetic eye drops can be used to decrease the pain.

VIRAL

Viral conjunctivitis is often associated with an infection of the upper respiratory tract, a common cold, and/or a sore throat. Its symptoms include excessive watering and itching. The infection usually begins with one eye, but may spread easily to the other.

2.2 INTRODUCTION OF MICROEMULSION [11]

Microemulsion have unique physical properties. They are composed of water, oil and a mixture of surfactants making a homogeneous, optically isotropic and thermodynamically stable solution. Microemulsions can be sterilized by filtration and their production is relatively simple and inexpensive. Because of these properties, they have attracted a great interest as drug delivery vehicles. Microemulsions can be applied as liquid membrane carriers to transport lipophilic substances through an aqueous medium or to carry hydrophilic substances across lipoidal medium. They are proposed for oral, topical, dermal, transdermal, parentenal and pulmonary administration of drugs. Although microemulsions have been known for a long period, their potential as vehicles for topical ocular drug delivery has been investigated only within the last decade. Preparing a pharmaceutical acceptable dosage form demands a clear understanding of the microemulsion structure, phase behaviour, factors leading to its thermodynamic stability, factors influencing drug release from the formulation, requirements of ideal microemulsion excipients, and the potential uses and limitations of the microemulsion system.

2.2.1 THEORIES OF MICROEMULSION FORMATION [12]

Historically, three approaches have been used to explain microemulsion formation and stability. They are as follows-

- Interfacial or mixed film theories.
- Solubilization theories.

• Thermodynamic treatments.

The free energy of microemulsion formation can be considered to depend on the extent to which surfactant lowers the surface tension of the oil water interface and change in entropy of the system such that,

$$Gf = \gamma a - T S$$

Where, Gf = Free energy of formation

A = Change in interfacial area of microemulsion

S = Change in entropy of the system , T = temperature

γ = Surface tension of oil water interphase

It should be noted that when a microemulsion is formed the change in A is very large due to the large number of very small droplets formed. In order for a microemulsion to be formed (transient) negative value of was required, it is recognized that while value of is positive at all times, it is very small and it is offset by the entropic component. The dominant favourable entropic contribution is very large dispersion entropy arising from the mixing of one phase in the other in the form of large number of small droplets. However there are also expected to be favourable entropic contributions arising from other dynamic processes such as surfactant diffusion in the interfacial layer and monomer-micelle surfactant exchange. Thus a negative free energy of formation is achieved when large reductions in surface tension are accompanied by significant favourable entropic change. In such cases, microemulsion is spontaneous and the resulting dispersion is thermodynamically stable.

2.2.2 COMPONENTS OF MICROEMULSION FORMULATIONS [13]

A large number of oils and surfactants are available which can be used as components of microemulsion systems but their toxicity, irritation potential and unclear mechanism of action limit their use. One must choose materials that are biocompatible, non-toxic, clinically acceptable, and use emulsifiers in an appropriate concentration range that will result in mild and non-aggressive microemulsions. The emphasis is, therefore, onthe use of generally regarded as safe (GRAS) excipients.

OIL PHASE

The oil component influences curvature by its ability to penetrate and swell the tail group region of the surfactant monolayer. As compare to long chain alkanes, short chain oil penetrate the tail group region to a greater extent and resulting in increased negative curvature (and reduced effective HLB). Following are the different oil are mainly used for the formulation of microemulsion:

- Saturated fatty acid-lauric acid, myristic acid, capric acid
- Unsaturated fatty acid-oleic acid, linoleic acid, linolenic acid
- Fatty acid ester-ethyl or methyl esters of lauric, myristic and oleic acid.

The main criterion for the selection of oil is that the drug should have high solubility in it. This will minimize the volume of the formulation to deliver the therapeutic dose of the drug in an encapsulated form.

SURFACTANT

The role of surfactant in the formulation of microemulsion is to lower the interfacial tension which will ultimately facilitates dispersion process during the preparation of microemulsion and provide a flexible around the droplets. The surfactant should have appropriate lipophilic character to provide the correct curvature at the interfacial region. Generally, low HLB surfactants are suitable for w/o microemulsion, whereas high HLB (>12) are suitable for o/w microemulsion. Following are the different surfactants are mainly used for microemulsion- Polysorbate (Tween 80 and Tween 20), Lauromacrogol 300, Lecithins, Decyl polyglucoside (Labrafil M 1944 LS), Polyglyceryl-6-dioleate (Plurol Oleique), Dioctyl sodium sulfosuccinate (Aersol OT), PEG-8 caprylic/capril glyceride (Labrasol).

COSURFACTANT

Co surfactants are mainly used in microemulsion formulation for following reasons:

- They allow the interfacial film sufficient flexible to take up different curvatures required to form microemulsion over a wide range of composition.

- Short to medium chain length alcohols (C3-C8) reduce the interfacial tension and increase the fluidity of the interface. Surfactant having HLB greater than 20 often require the presence of cosurfactant to reduce their effective HLB to a value within the range required for microemulsion formulation.

2.2.3 PREPARATION OF MICROEMULSION [14]

Following are the different methods are used for the preparation of microemulsion

- Phase titration method
- Phase inversion method

Phase titration method Microemulsions are prepared by the spontaneous emulsification method (phase titration method) and can be portrayed with the help of phase diagram. As quaternary phase diagram (four component system) is time consuming and difficult to interpret, pseudo ternary phase diagram is constructed to find out the different zones including microemulsion zone, in which each corner of the diagram represents 100% of the particular components. Pseudo-ternary phase diagrams of oil, water, and co-surfactant/surfactants mixtures are constructed at fixed cosurfactant/surfactant weight ratios. Phase diagrams are obtained by mixing of the ingredients, which shall be pre-weighed into glass vials and titrated with water and stirred well at room temperature. Formation of monophasic/ biphasic system is confirmed by visual inspection. In case turbidity appears followed by a phase separation, the samples shall be considered as biphasic. In case monophasic, clear and transparent mixtures are visualized after stirring; the samples shall be marked as points in the phase diagram. The area covered by these points is considered as the microemulsion region of existence.

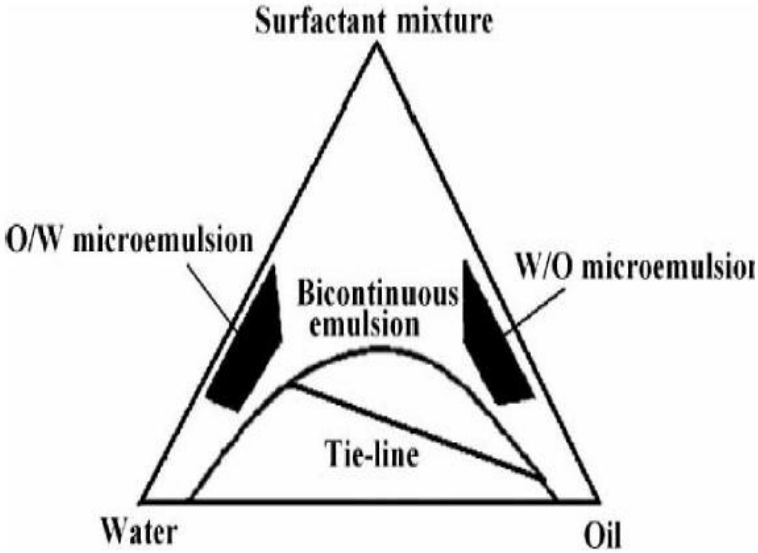


Figure 2.4: Ternary Phase Diagram

2.2.4 ADVANTAGES OF MICROEMULSION OVER OTHER DOSAGE FORMS [15]

- Increase the rate of absorption
- Eliminates variability in absorption
- Helps solublize lipophilic drug
- Provides a aqueous dosage form for water insoluble drugs
- Increases bioavailability
- Various routes like tropical, oral and intravenous can be used to deliver the product Rapid and efficient penetration of the drug moiety
- Helpful in taste masking
- Provides protection from hydrolysis and oxidation as drug in oil phase in o/w microemulsion is not exposed to attack by water and air.
- Liquid dosage form increases patient compliance
- Less amount of energy requirement

2.2.5 DISADVANTAGES OF MICROEMULSION BASED SYSTEMS [16]

1. Use of a large concentration of surfactant and co-surfactant is necessary for stabilizing the droplets of microemulsion.
2. Limited solubilizing capacity for high-melting substances used in the system.
3. The surfactant should be nontoxic for use in pharmaceutical applications

2.2.6 LIMITATIONS

Some factors limit the use of microemulsion in pharmaceutical applications.

1. The need of pharmaceutically acceptable ingredients limits the choice of microemulsion components (e.g., oil, surfactant and cosurfactants) leading to difficulties in formulation.

2. The concentration of surfactants and co-surfactants used must be kept low for toxicological reasons.

3. Microemulsion also suffers from limitations of phase separation.

For intravenous use, the demand of toxicity on the formulation is rigorous and very few studies have been reported so far.

The major limitation is the toxicity of excipients i.e. surfactant/ co-surfactants. Exploration of safe excipients and evaluation of the toxicity parameters of available excipients may help in further expansion of research in this field.

2.2.7 APPLICATIONS OF MICROEMULSION [14]

1. Parenteral delivery.
2. Oral drug delivery.
3. Topical drug delivery.
4. Ocular and pulmonary delivery.
5. Microemulsion in biotechnology.

2.3 IN SITU GELLING SYSTEM

2.3.1 INTRODUCTION

A more desirable dosage form would be one that can deliver drug in a solution form, create little to no problem of vision and need be dosed no more frequently than once or twice daily. In situ activated gel- forming systems are those which are when exposed to physiological conditions will shift to a gel phase.

This new concept of producing a gel in situ was suggested for the first time in the early 1980s. Gelation occurs via the cross-linking of polymer chains that can be achieved by covalent bond formation (chemical cross-linking) or non-covalent bond formation (physical cross-linking).

The progress that has been made in gel technology is in the development of a droppable gel. In situ gel-forming systems can be described as low viscosity solutions that undergo phase transition in the conjunctival cul-de-sac to form viscoelastic gels due to conformational changes of polymers in response to the physiological environment.The rate of in situ gel formation is important because between instillation in the eye and before a strong gel is formed, the solution or weak gel is produced by the fluid mechanism of the eye.

2.3.2 IMPORTANCE

The major importance is the possibility of administrating accurate and reproducible quantities compared to already formed gel. It is conveniently dropped as a solution into the conjunctival sac, enhancing patient compliance and minimizing interference with blinking. It increases the contact time of drug with the mucus at the site of absorption and has better bioavailability.

2.3.3 STIMULI SENSITIVITY OF HYDROGELS 1 [17, 18]

Table 2.1 Stimuli Sensitivity of Hydrogels

External stimuli	Mechanism	Example
Temperature	Formulation is liquid at room temperature(20-25˚c) which undergoes gelation in contact with body fluid(35- 37˚c) •Temperature increases degradation of polymer chains which leads to formation of hydrophobic domains & transition of an aqueous liquid to hydrogel network	Poloxamer Polyester Cellulose derivatives Xyloglucan
Ionic interaction	Formulation undergoes liquid-gel transition under influence of an increase in ionic strength • Gel formation takes place because of complexation with polyvalent cations (like Ca2+) in lacrimal fluid	Chitosan Gellan gum Alginate
pH	Sol to gel transition when pH raised from 4.2 to 7.4(eye pH) • At higher pH polymer forms hydrogen bonds with mucin which leads to formation of hydrogel network	Cellulose acetate phthalate (CAP) Pseudolatexes Polyox Acrylates (carbopol)

2.4 INTRODUCTION OF DRUG [19]

- **Ntamycin:**

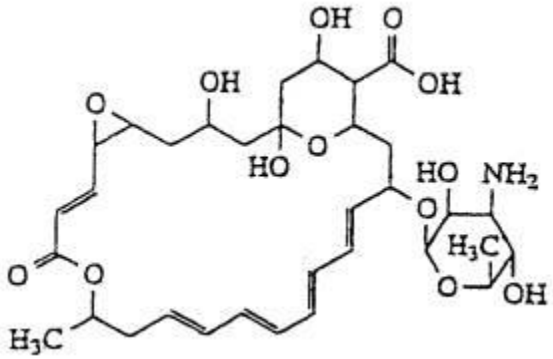
  Natamycin (Pimaricin) is an antifungal which can be used as antibiotic to treat most fungus infections. Natamycin is an natural antimicrobial food additive used to protect food from mold and yeast growth.

- **Registered Index:**
  - CAS**:** 7681-93-8

- **Structure & Characteristic**

  Molecular formula : C33 H47 NO13

  Molecular wheight : 665.67



- **Appearance**:

  White to yellow crystalline powder. No taste or odour

- **Microbiologic Character:**

  Natamycin can effectively inhibit the growth of most mould and yeast, but work resultless in hindering the growth of some microbes like bacteria and virus. Generally speaking, 1-10mg/kg , Natamycin is effective enough to inhibit the growth of mould and yeast yet the application amount of sorbic acid is 500mg/kg.

- **Solubility and stability :**

  Natamycin have low solubility in water, higher alcohols, ether and ester, slightly soluble in methanol, completely soluble in glacial acetic acid and

dimethylsulfoxide.

--sensitive to oxidant and ultraviolet radiation

--meltingpoint280℃(decomposed)

The products performs well between the pH range of 5 and 7 with only little reduction in activity. They remains stable at ambient temperature and are unaffected by short periods of exposure to temperatures as high as 100℃ but must be protected from exposure to direct sunlight. Natamycin are extremely stable under dry condition. However, the stability can be greatly affected by PH value, temperature, daylight, oxidant and heavy metal content. Suggested PH value: 4-7. Avoided from direct sunlight exposure and high temperature.

- **Antimicrobial Property :**

  Natamycin binds to and alters the fungal cell membrane so that vital structures inside the cell pass though the membrane and out of the cell. Without these structures, the fungal cells cannot survive.

- **Safety:**

  Animal studies on rabbits, dogs, and cows indicate that natamycin has no toxic effects even at high levels of ingestion. In addition, natamycin was found to have no reproductive or mutagenic qualities. Animal studies also show that natamycin is fat and water insoluble, allowing an estimated 90% to be excreted through normal gastrointestinal functions. Natamycin is approved for use by the FDA and has been widely used in Europe for over 35 years. Natamycin is an approved food additive, but regulations regarding its usage differ from country to country. The joint FAO/WHO Expert Committee on Food Additives has set an acceptable daily intake of 0.3 mg per kg of body weight per day.

- **Method of production:**

  Natamycin is made from streptomyces natalensis through deep fermenting and complicated extracting process. Natamycin product is usually made of 50% Natamycin mixed with lactose or glucose.

- **Application:**

  Primary applications :

  Natamycin can be used in a variety of foods and beverages:

  · Cheese , Surface treatment for cheeses

  · Baked food

  · Meat , Jam, Jelly, Marinated food, Fish, Chicken

  · Surface treatment for semi-dried, cured meat products

  · Drinks , Juice , Wines

  · Yogurts , man-made butter

- **Functions:**

  Natamycin are effective anti-microbial agents. Natamycin as food preservatives can:

  · enhance the quality of food product, and significantly extend the shelf life of foods by preventing yeast and mould spoilage.

- **Storage**:

  Sealed and kept in cool place where there is no direct sunshine exposure and it is better for temperature is below 15ºC.

- **Specification:**

Table 2.2: Specification of Natamycin

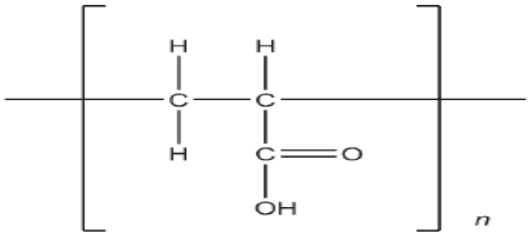
Item	**Standard Requirement**	**Result**
Appearance	White to yellow crystalline powder	Conform
Purity	≥50%	51.2%
Moisture	6.0-9.0%	6.7%

PH	5.0-7.5	6.0
Specific Rotation	+2760 ~ +2800	+2780
Ash	≤0.5%	0.2%
Heavy metals	< 20ppm	< 10ppm
Pb	< 20ppm	< 5ppm
Arsenic (As)	< 3ppm	< 3ppm
Hg	< 1ppm	< 1ppm
Microbiological Count	< 10cfu/g	< 10cfu/g
Pathogen	Absent	Absent
E. coli	Negative/in 25g	Negative/in 25g
Salmonella	Negative/in 25g	Negative/in 25g

2.5 INTRODUCTION OF EXCIPIENT [20] :

Carbopol 940:

- **Non proprietary names:**
  - USPNF: Carbomer
  - BP: Carbomer
  - PhEur: Carbomer
- **Functional categories:**
  - NF : Gelling agent.
  - Others: Bioadhesive material; controlled-release agent; emulsifying agent, agent; suspending agent; tablet binder.
- **Synonym:**
  - Acrypol;
  - Acritamer; acrylic acid polymer;
  - Carbomer;
  - Carboxy polymethylene;
  - Polyacrylic acid;
  - Carboxyvinyl polymer.
- **Chemical names:** Carbomer
- **CAS Registry Number:** 9003-01-4
- **Structural formula:**



Acrylic acid monomer unit in carbomer polymers.

Carbomer polymers are formed from repeating units of acrylic acid. The monomer unit is shown above. The polymer chains are crosslinked with allyl sucrose or allyl pentaerythritol.

- **Description:**

Carbomers are white-colored, 'fluffy', acidic, hygroscopic powders with a characteristic slight odor.

- **Typical properties:**

  - Melting point**:** Decomposition occurs within 30 minutes at 260°C.
  - Moisture content**: N**ormal water content is up to 2% w/w. However, carbomersare hygroscopic and typical equilibrium moisture content at 25°C and 50% relative humidity is 8–10% w/w.
  - Viscosity: 3000 CP
  - pH**:** 2.5 – 4 (0.2% dispersion)

- **Solubility:**

Soluble in water and, after neutralization, in ethanol (95%) and glycerine.

- **Stability and Storage Conditions:**

Carbomers are stable, hygroscopic materials that may be heated at temperatures below 104°C for up to 2 hours without affecting their thickening efficiency. However, exposure to excessive temperatures can result in discoloration and reduced stability. Carbomer powder should be stored in an airtight, corrosion-resistant container in a cool, dry place.

- **Safety :**

Carbomers are used extensively in non-parenteral products, particularly topical liquid and semisolid preparations. They may also be used in oral formulations, although only certain grades can be used. Carbomers are generally regarded as essentially nontoxic and non-irritant materials; there is no evidence in humans of hypersensitivity reactions to carbomers used topically.

- **Applications in pharmaceutical formulation technology:**

Carbomers are used in liquid or semisolid pharmaceutical formulations as rheology modifiers. Formulations include creams, gels, lotions and ointments for use in ophthalmic,(5–7) rectal,(8–10)topical(11–20) and vaginal(21,22) preparations.

Table 2.3 : use of carbomer

Use of carbomer	
Use	Concentration (%)
Emulsifying agent	0.1-0.5
Gelling agent	0.5-2.0
Suspending agent	0.5-1.0
Controlled release agent	5.0-30.0

Hydroxypropylmethylcellulose (HPMC)

- **Introduction:**

Hydroxypropylmethylcellulose is cellulose having some of the hydroxyl groups in the form of the methyl ether and some in the form of the 2-hydroxypropyl ether. The various grades commercially available are distinguished by a number indicative of the apparent viscosity in millipascal seconds of a 2% w/v solution measured at 20 ^{o}C. The general molecular structure can be as follow.

- **Non proprietary name:**
  - BP : Hypromellose
  - JP : Hydroxyl propyl methyl cellulose
  - PhEur : Hypromellosum
  - USP NF : Hypromellose

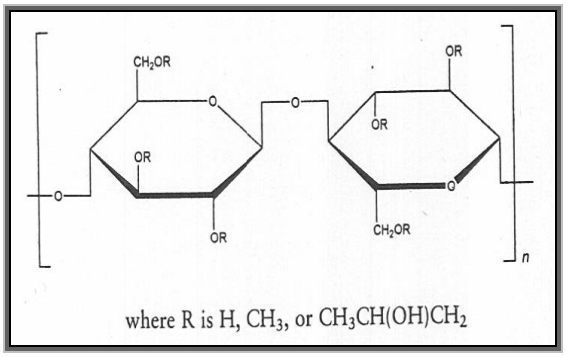
- **Description:**

Odorless, tasteless, white to creamy white fibrous and granule powder.

- **Chemical name**:

Cellulose, 2- hydroxypropyl methyl ether

- **Structural formula**:



- **Molecular weight:** 10, 000 to 15, 00, 000
- **Functional categories:**
  - Coating agent, film former,
  - rate controlling polymer for sustained release,
  - stabilizer,
  - suspending agent,
  - tablet binder,
  - Viscosity-increasing agents.

- **Typical properties:**
  - pH : 5.5 - 8 (1% aqueous solution at 25 °C)
  - Bulk density : 0.341 gm/ml
  - Tapped density : 0.557 gm/ml
  - Moisture content : 5-10%
  - Melting point : 190-200^{0}C
  - Solubility : Soluble in cold water, forming a viscous colloidal solution. Practically insoluble in chloroform, ethanol, and ether. But soluble in mixture of ethanol and dichloromethane, and mixture of water and alcohol.

- **Stability and storage condition:**

It is stable, although it is hygroscopic after drying. But it should be Store in well closed container in cool and dry place. Solution are stable at pH 3-11.increase the temp reduce the viscosity. It undergoes sol-gel transformation on heating and cooling. The gel point is 50-90.

- **Incompatibilities:**

Incompatible with oxidizing agent:

Table 2.3: Typical viscosity values for 2% (w/v) aqueous solution of Methocel

HPMC grades	Viscosity (mPas)
K100LVP	80-120
K4M	3000-5000
K15MP	12000-21000
K100MP	80000-120000
E4M	3500-5600
E100MPCR	8000-13000
E3 PREMLV	2.4-3.6
E5 PREMLV	4-6
E6 PREMLV	5-7
E15 PREMLV	12-18
E50 PREMLV	40-60
K3 PEMLV	2.4-3.6

HPMC is a stable material, although it is hygroscopic after drying. Solutions are stable at pH 3-11 increasing temperature reduces the viscosity of solutions. HPMC undergoes a reversible sol-gel transformation upon heating and cooling, respectively. The gel point is 50-90 ^{o}C, depending upon the grade and concentration of material.

HPMC absorbs moisture from the atmosphere, the amount of water absorbed depending upon the initial moisture content and the temperature and relative humidity of the surrounding air. So, the HPMC powder should be stored in a well-closed container, in a cool, dry place.

HPMC is incompatible with some oxidizing agents. Since it is nonionic, HPMC will not complex with metallic salts or ionic organics to form insoluble precipitates. HPMC dust may be irritating to the eyes and eye protection is recommended. Excessive dust generation should be avoided to minimize the risks of explosion. HPMC is combustible.

- **Application of HPMC in pharmaceutical formulation and technology:**

HPMC is widely used in oral and topical pharmaceutical formulations. In oral products, HPMC is primarily used as a tablet binder, in film coating, and as an extended release tablet matrix. Concentration between 2% and 5% w/w may be used as a binder in either wet or dry granulation processes. High viscosity grades may be used to retard the release of drugs form a matrix at levels of 10 – 80% w/w in tablets and capsules. Depending upon the viscosity grade, concentrations of 2 – 20% w/w are used for film forming solutions to film coat tablets. Lower viscosity grades are used in aqueous film coating solutions, while higher viscosity grades are used with organic solvents. HPMC is also used as a suspending and thickening agent in topical formulations, particularly ophthalmic preparations. Compared with methylcellulose, HPMC products solutions of greater clarity, with fewer undispersed fibers present, and is therefore preferred in formulations for ophthalmic use. HPMC at concentrations between 0.45 – 1.0 % w/w may be added as a thickening agent to vehicles for eye drops and artificial tear solutions. HPMC is also used as an emulsifier, suspending agent, and stabilizing agent in topical gels and ointments. As a

protective colloid, it can prevent droplets and particles from coalescing or agglomerating, thus inhibiting the formation of sediments. In addition, HPMC is used in the manufacture of capsules, as an adhesive in plastic bandages, and as a wetting agent for hard contact lenses. It is also widely used in cosmetics and food products.

TWEEN 80 [20]

- **Non-proprietary Names**

  BP: Polysorbate 80

  PhEur: Polysorbate 80

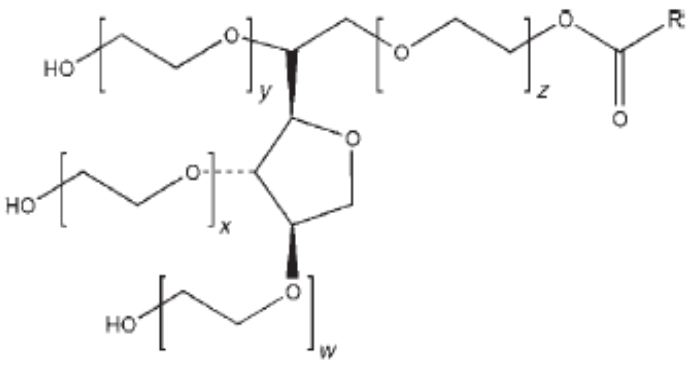
  USP-NF: Polysorbate 80

- **Synonyms :** polysorbate

- **Chemical Name and CAS Registry Numbers:**

  Polyoxyethylene 20 sorbitan mmonooleate and [9005-65-6]

- **Structural formula**: C64H124O26



- **Molecular Weight**: 1310

- **Functional Category:**

- Dispersing agent; emulsifying agent; nonionic surfactant; solubilising agent; suspending agent; wetting agent.

- **Applications in Pharmaceutical Formulation or Technology**

Table 2.4: Use of Polysorbate

USE OF POLYSORBATE	
Use	Concentration (%)
Emulsifying agent	1-15
Sulubilizing agent	1-15
Wetting agent	0.1-3

Description

- Polysorbates have a characteristic odor and a warm, somewhat bitter taste.

- **Typical Properties**:
  - **Acidity/alkalinity pH** : 6.0–8.0 for a 5% w/v aqueous solution.
  - **Flash point** : 1498C
  - **HLB value :** 15
  - **Hydroxyl value** : 65-80
  - **Moisture content** : 3.00.
  - **Surface tension :** 42.5

- **Stability and Storage Conditions:**

Polysorbates are stable to electrolytes and weak acids and bases; gradual saponification occurs with strong acids and bases. The oleic acid esters are sensitive to oxidation. Polysorbates are hygroscopic and should be examined for water content prior to use and dried if necessary. Also, in common with other polyoxyethylene surfactants, prolonged storage can lead to the formation of peroxides.

- **Incompatibilities:**

Discoloration and/or precipitation occur with various substances, especially phenols, tannins, tars, and tarlike materials. The antimicrobial activity of paraben preservatives is reduced in the presence of polysorbates

- **Safety:**

Polysorbates are widely used in cosmetics, food products, and oral, parenteral and topical pharmaceutical formulations, and are generally regarded as nontoxic and nonirritant materials. There have, however, been occasional reports of hypersensitivity to polysorbates following their topical and intramuscular use. Polysorbates have also been associated with serious adverse effects, including some deaths, in low-birth weight infants intravenously administered a vitamin E preparation containing a mixture of polysorbates 20 and 80.When heated to decomposition, the polysorbates emit acrid smoke and irritating fumes.

The WHO has set an estimated acceptable daily intake for polysorbates 20, 40, 60, 65, and 80, calculated as total polysorbate esters, at up to 25 mg/kg body-weight.

POLYETHYLENE GLYCOL [20]:

- **Nonproprietary Names:**

BP: Macrogols

PhEur: Macrogol 400

USP-NF: Polyethylene Glycol

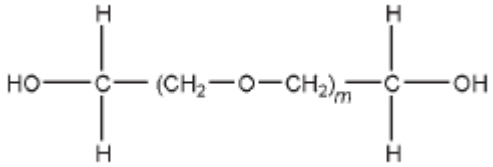
- **Synonyms :**

Carbowax; Carbowax Sentry; Lipoxol; Lutrol E; macrogola; PEG; Pluriol E; polyoxyethylene glycol.

- **Chemical Name and CAS Registry Numbers**

a-Hydro-o-hydroxypoly(oxy-1,2-ethanediyl) [25322-68-3]

- **Structural formula**:



- **Molecular Weight**: 380-420

- **Functional Category:**

  Ointment base; plasticizer; solvent; suppository base; tablet and capsule lubricant

- **Applications in Pharmaceutical Formulation or Technology :**

Polyethylene glycols (PEGs) are widely used in a variety of pharmaceutical formulations, including parenteral, topical, ophthalmic, oral, and rectal preparations. Polyethylene glycol has been used experimentally in biodegradable polymeric matrices used in controlled-release systems. Polyethylene glycols are stable, hydrophilic substances that are essentially nonirritant to the skin; They do not readily penetrate the skin, although the polyethylene glycols are water-soluble and are easily removed from the skin by washing,making them useful as ointment bases.Solid grades are generally employed in topical ointments, with the consistency of the base being adjusted by the addition of liquid grades of polyethylene glycol.

- **Description** :

  The USP32–NF27 describes polyethylene glycol as being an addition polymer of ethylene oxide and water. Polyethylene glycol grades 200–600 are liquids; grades 1000 and above are solids at ambient temperatures. Liquid grades (PEG 200–600) occur as clear, colorless or slightly yellow-colored, viscous liquids. They have a slight but characteristic odour and a bitter, slightly burning taste. PEG 600 can occur as a solid at ambient temperatures.

- **Typical Properties**
  - **Density** : 1.11-1.114 g/cm3
  - **Flash point** : 238 ºC

- **HLB value :** 15
- **Hydroxyl value** : 264-380
- **Freezing point** : 4-8 º c
- **Viscosity :** 94-116

- **Stability and Storage Conditions**

  Polyethylene glycols are chemically stable in air and in solution, although grades with a molecular weight less than 2000 are hygroscopic. Polyethylene glycols do not support microbial growth, and they do not become rancid. Polyethylene glycols and aqueous polyethylene glycol solutions can be sterilized by autoclaving, filtration, or gamma irradiation.

- **Incompatibilities:**

  The chemical reactivity of polyethylene glycols is mainly confined to the two terminal hydroxyl groups, which can be either esterified or etherified. However, all grades can exhibit some oxidizing activity owing to the presence of peroxide impurities and secondary products formed by autoxidation. Liquid and solid polyethylene glycol grades may be incompatible with some colouring agents.

- **Safety:**

  Polyethylene glycols are widely used in a variety of pharmaceutical formulations. Generally, they are regarded as nontoxic and nonirritant materials. Adverse reactions to polyethylene glycols have been reported, the greatest toxicity being with glycols of low molecular weight. However, the toxicity of glycols is relatively low. Polyethylene glycols administered topically may cause stinging, especially when applied to mucous membranes. Hypersensitivity reactions to polyethylene glycols applied topically have also been reported, including urticaria and delayed allergic reactions.

Chapter 3
Literature Review

LITERATURE REVIEW:

MICROEMULSION FOR OCULAR DELIVERY

1. **Gasco et al** [21] developed timolol as an ion-pair with octanoate was achieved by use of an oil-in-water microemulsion containing lecithin as a surfactant. The microemulsion, a solution of the ion-pair and a solution of timolol alone were instilled in the conjunctival sac of rabbits. The bioavailability of timolol from the microemulsion and the ion-pair solution was higher than that obtained from timolol alone. The areas under the curve for timolol in aqueous humour after administration of the microemulsion and the ion-pair solution were 3.5 and 4.2 times higher, respectively, than that observed after the administration of timolol alone.

2. **Lin et al** [22]**:** formulated microemulsion of chloramphenicol using Span20 + Tween20 isopropyl myristate (IPM) + H2O. Chloramphenicol in the common eye drops hydrolyzes easily. The main product of the hydrolysis is glycol. Here, the chloramphenicol was trapped into the oil-in-water (o/w) microemulsions free of alcohols. Its stability was investigated by the high performance liquid chromatography (HPLC) assays in the accelerated experiments of 3 months. The results of HPLC revealed that the content of the glycols in the microemulsion formulation was much lower than that in the commercial eye drops at the end of the accelerated experiments.

3. **Yong et al** [23]: prepared microemulsion of dexamethasone for topical ocular drug delivery. Microemulsion developed system showed an acceptable physicochemical behaviour and presented good stability for 3 months. The ocular irritation test used suggested that the microemulsion did not provide significant alteration to eyelids, conjunctiva, cornea and iris. This formulation showed greater penetration of dexamethasone in the anterior segment of the eye and also release of the drug for a longer time when compared with a conventional preparation. The area under the curve obtained for the

microemulsion system was more than two fold higher than that of the conventional preparation ($P < 0.05$).

4. **Habe et al** [24] developed water-continuous microemulsions for ocular application were developed and the physico-chemical parameters characterized. These microemulsions have favourable features for ocular use. They show an acceptable physicochemical behaviour, especially pH value, refractive index and viscosity, and a good physiological compatibility. A prolonged pilocarpine release from the microemulsions with lecithin was shown in in vitro experiments. The miotic activity was measured on albino rabbits. For ophthalmological use the miotic retard effect of pilocarpine in microemulsions turns out to be advantageous.

5. **Radomska-Soukharevet et al** [25]: developed microemulsion systems, both o/w and w/o, composed of isopropyl myristate, soybean lecithin (Epikuron 200), Polysorbate 80, Cremophor EL, n-butanol and triacetine, taken at various amounts and in various combinations, were tested in order to assess their physical-chemical properties. Some ingredients of the microemulsions were physiologically acceptable, except one formulation containing n-butanol used as a model reference system. Phase diagrams were made for all microemulsions studied in order to test the influence of components on the boundaries of the stable microemulsion domains. The greatest area of stable microemulsion system was obtained for the formulation in which n-butanol was used, while the smallest area was obtained when soybean lecithin (Epikuron 200) was used as a surfactant. Microemulsions stored at 25 degrees C for the period up to 12 months, showed physical changes depending on ingredients. The study made it possible to select the most stable microemulsion system meeting the requirements of eye drops**.**

IN SITU GEL FOR OCULAR

1. **Charoo et al** [26] : developed Sol to gel system of ciprofloxacin hydrochloride was prepared utilizing the phase transition properties of HPMC K15M and carbopol 934. Concentration in aqueous humor was determined and stability studies were carried out as per the ICH guidelines. The sol to gel system exhibited a zero order drug release pattern over 24 h in vitro release studies. The drug was active against selected microorganisms in the microbial efficacy studies. Better improvement in artificially induced bacterial conjunctivitis in rabbit's was observed in animals treated with the sol to gel system compared with marketed eye drops. Drug concentration in aqueous humor was greater than the minimum inhibitory concentration (MIC 90) against selected microorganisms. The shelf life of product was more than 2 years.

2. **Jain et al** [27]: prepared and evaluation of a once a day ophthalmic delivery system for ciprofloxacin hydrochloride based on the concept of pH-triggered in situ gelation. The in situ gelling system involved the use of polyacrylic acid (Carbopol 980NF) as a phase transition polymer, hydroxyl propyl methylcellulose (Methocel K100LV) as a release retardant, and ion exchange resin as a complexing agent. Ciprofloxacin hydrochloride was complexed with ion exchange resin to avoid incompatibility between drug and polyacrylic acid. The developed formulation was stable and nonirritant to rabbit eyes and in vitro drug release was found to be around 98% over a period of 24 hours.

3. **Edsman et al** [28] study the ocular residence time of carbomer gels have been monitored in humans and the gels were also rheologically characterised. The contact time of Carbopol 974P and Carbopol 1342NF was concentration dependent and was approximately 2-2.5 h for a 2°/, gel. There was a good correlation of the human contact time and the elastic properties of the gels. The miotic response from gels with pilocarpine nitrate was monitored in rabbits and the area under the curves (AUCs') were calculated. The relative

AUCs were 1.5-2.1 for the gel preparations relative to a solution. No concentration dependence was seen and there was no significant difference in AUC between the different carbomers. The ocular contact time of the gels were much less in rabbits than those obtained in humans.

4. **Srividya et al** [29] formulate and evaluate of an ophthalmic delivery system of an antibacterial agent, ofloxacin, based on the concept of pH-triggered in situ gelation. Polyacrylic acid (Carbopol 940) was used as the gelling agent in combination with hydroxypropylmethylcellulose (Methocel E50LV) which acted as a viscosity enhancing agent. The developed formulation was therapeutically efficacious, stable, non-irritant and provided sustained release of the drug over an 8-h period. The developed system is thus a viable alternative to conventional eye drops.

5. **Liu et al** [30] formulate and evaluation of an ophthalmic delivery system containing an antibacterial agent, enoxacin, based on the concept of ophthalmic sustained gel, in which 2 hydroxypropyl-beta-cyclo-dextrin (HP-b-CD) was used as a penetration enhancer in combination with hydroxypropylmethylcellulose (Methocel F4M) which acted as a vehicle. The developed formulation was therapeutically efficacious, nonirritant, and provided sustained release of the drug over 8 h period in vitro and 7 h period in vivo. The developed system is a viable alternative to conventional eye drops.

6. **Abraham *et al*** [31] investigated that the poor bioavailability and therapeutic response exhibited by conventional ophthalmic solutions due to rapid pre-corneal elimination of the drug may be overcome by the use of *in situ* gel forming systems that are instilled as drops into the eye and then undergo a sol-gel transition in the cul-de-sac. The present work describes the formulation and evaluation of an ophthalmic delivery system of an antibacterial agent ofloxacin, based on the concept of ion-activated *in situ* gelation. Sodium

alginate was used as the gelling agent in combination with HPC (Hydroxy Propyl Cellulose) that acted as a viscosity-enhancing agent. *In vitro* release studies indicated that the alginate/HPC solution retained the drug better than the alginate or HPC solutions alone. The formulations were therapeutically efficacious, sterile, stable and provided sustained release of the drug over a period of time. These results demonstrate that the developed system is an alternative to conventional ophthalmic drops, patient compliance, industrially oriented and economical.

7. **Qi et al** [32] developed a thermosensitive in situ gelling and mucoadhesive ophthalmic drug delivery system containing puerarin based on poloxamer analogs (21% (w/v) poloxamer 407/5% (w/v) poloxamer 188) and carbopol (0.1% (w/v) or 0.2% (w/v) carbopol 1342P NF). The combined solutions would convert to firm gels under physiological condition and attach to the ocular mucosal surface for a relative long time. The incorporation of carbopol 1342P NF not only did not affect the pseudoplastic behavior with hysteresis of the poloxamer analogs solution and leaded to a higher shear stress at each shear rate, but also enhanced the mucoadhesive force significantly. In vitro release studies demonstrated diffusion-controlled release of puerarin from the combined solutions over a period of 8 h. In vivo evaluation (the elimination of puerarin in tear and intraocular pressure-lowering effect) indicated the combined solutions had better ability to retain drug than poloxamer analogs or carbopol alone. It appears that ocular bioavailability can be increased more readily by using the in situ gelling and mucoadhesive vehicle.

8. **Lin et al** [33] developed in situ gell of 0.3% carbopol and 14% pluronic solutions showed a significant enhancement in gel strength in the physiological condition and the in vitro release and in vivo pharmacological studies indicated that the carbopol / pluronic solution had the better ability to retain drug than the carbopol or pluronic solutions alone. The results

demonstrated that the carbopol / pluronic mixture can be used as an in situ gelling vehicle to enhance the ocular bioavailability,.

9. **Sultana et al** [34] developed carbopol-Methyl cellulose based sustained-released ocular delivery system for pefloxacin mesylate using rabbit eye model. The results demonstrated that the carbopol/methyl cellulose mixture could be used by an *In-situ* gelling vehicle to enhance the ocular bioavailability of pefloxacin mesylate.

MICROEMULSION BASED IN SITU GEL FOR OCULAR

1. **Zhu et al** [35] developed a novel microemulsion in situ electrolyte-triggered gelling system for ophthalmic delivery of a lipophilic drug, cyclosporine A (CsA). A CsA-loaded microemulsion was prepared using castor oil, Solutol HS 15 (surfactant), glycerol andwater. This microemulsionwas then dispersed in a Kelcogel® solution to form the final microemulsion in situ electrolyte-triggered gelling system. In vitro, the viscosity of the CsA microemulsion Kelcogel® system increased dramatically on dilution with artificial tear fluid and exhibited pseudo-plastic rheology. In vivo results revealed that the AUC0→32h of corneal CsA for the microemulsion Kelcogel® system was approximately three-fold greater than for a CsA emulsion. Moreover, at 32 h after administration, CsA concentrations delivered by the microemulsion Kelcogel® system remained at therapeutic levels in the cornea. This CsA microemulsion in situ electrolytetriggered gelling system might provide an alternative approach to deliver prolonged precorneal residence time of CsA for preventing cornea allograft rejection.

2. **Ma et al** [36] prepared a novel ocular cationic microemulsion-in situ gel (CM-ISG) system with vitamin A palmitate (VAP) as model drug, and investigate the corneal retention behavior and corneal irritation of the system. VAP/CM was prepared by a process based on supply of energy, and the before-and-after gelation rheology of VAP/CM-ISG was investigated. In vitro VAP release and gel dissolution of both VAP/CM-ISG and Oculotect Gel was determined. And in vitro corneal retention behavior of both formulations was evaluated by captive bubble technique. Ocular irritation test was carried out based on the Draize method. Images of TEM showed that homogenous VAP/CM was made, and no significant differences of particle size were found between the VAP/CM and VAP/CM in Poloxamer 407 gel. Rheology study illustrated that VAP/CM reduced the phase transition temperature of Poloxamer 407 gel by 1.5 degrees C, and the elastic modulus increased about 15.7 times. The in vitro

release and gel dissolution profile of both formulations exhibited the characteristics of zero order kinetics. Comparing with Oculotect Gel, desorption kinetics study of VAP/CM-ISG exhibited longer corneal retention time and smaller contact angle. Irritation test showed a good ocular compatibility of VAP/CM-ISG. Therefore, VAP/CM-ISG combined both advantages of the cationic microemulsion and in situ gel system, provided better wettability and longer ocular retention time. It might be a promising ocular drug delivery system.

NATAMYCIN FOR OCULAR

1. **Rajasekaran et al** [37] developed ocuserts using different polymers such as eudragit L-100, eudragit S-100, eudragit RL-100, hydroxy propyl methyl cellulose phthalate and cellulose acetate phthalate at various proportion and combinations using PEG-400 as plasticizer. The prepared ocuserts were evaluated for their physicochemical parameters like drug content, weight uniformity, folding endurance, thickness, % moisture absorption and water vapour transmission rate. The in vitro drug release from the formulations was studied using commercial semi permeable membrane and the in vitro release kinetic datas were treated according to the diffusion models proposed by Higuchi and Peppas in order to access the mechanism of drug release from the formulations, which were following zero order kinetics. All the formulations showed no change in the physical appearance and the FTIR studies indicated no possibility of interaction between drug and polymer. The expected zero order release for one day was observed in the formulation D1 (3% Eudragit RL100 and 1% Eudragit L100).

2. **Lalitha et al**. [38] studied the in vitro activity of natamycin against *Fusarium* and *Aspergillus* species isolated from corneal ulcers and compared the MICs obtained by using both pharmaceutical-grade NAT powder (NAT-P) and commercially available NAT eye drops (NAT-D). A total of 100 fungal isolates recovered from clinical cases of corneal ulcer were evaluated in the present study. Comparison of the MICs between NAT-P and NAT-D showed perfect agreement, with 92.6% for *Fusarium* spp. (38 of 41 isolates) and 71.9% for *A. flavus* (23 of 32 isolates). The present study shows that the NAT had good activity against both *Fusarium* and *Aspergillus* spp., with slightly higher NAT MICs for *Aspergillus* spp.

3. **Saleem et al** [39] studied efficacy of topical natamycin 5% was studied using a reproducible model of keratomycosis produced by *Candida albicans* in the rabbits. *Candida albicans* was isolated from infected human eye and 4 x 105

cells of the *Candida albicans* was injected into the corneal stroma of the eyes of 15 rabbits. All eyes developed a corneal ulcer without pretreatment with immunosuppressive agents. Forty-eight hours after inoculation, the animals were divided into two groups: test group I, 10 eyes receiving natamycin drops in a 5% suspension; control group II, five eyes receiving 0.9% normal saline solution. The rabbits' corneas were removed for *Candida albicans* recovery and placed in 1 ml of sterile 0.9% normal saline solution, minced within two hours with scalpel and thoroughly homogenized with a piston and mortar. Serial dilutions of this corneal solution from 10-1 – 10-4 were made in 0.9% sterile saline solution and 100 µl aliquots were plated onto tryptic soy agar. All cultures of cornea from the treated eyes were negative after seven days of inoculation while five cultures were still positive in the control eyes at the end of the experiment. It was found that 5% natamycin was effective in treating experimental *Candida albicans* induced keratomycosis in rabbits.

Chapter 4
Materials and Method

4.1 List of Materials :

MATERIALS	VENDOR'S NAME
Natamycin	Gifted by Sun Pharma
Tween® 20	Central Drug House Pvt. Ltd, India
Tween® 60	Central Drug House Pvt. Ltd, India
Tween® 80	Central Drug House Pvt. Ltd, India
Span® 20	Central Drug House Pvt. Ltd, India
Span® 80	Central Drug House Pvt. Ltd, India
PEG® 200	Central Drug House Pvt. Ltd, India
Propylene Glycol	Central Drug House Pvt. Ltd, India
PEG 400	Central Drug House Pvt. Ltd, India
Labrasol	Kindly gifted by Gattefosse, France
Transcutol	Kindly gifted by Gattefosse, France
Brij® 35	Central drug house Pvt. Ltd, India
Soluphor P (2-Pyrrolidone)	Kindly gifted by Gattefosse, France
Triacetin®	Central Drug House Pvt. Ltd, India
Oleic Acid	Central Drug House Pvt. Ltd, India
Isopropyl Myristate (IPM)	Central Drug House Pvt. Ltd, India
Castor Oil	Central Drug House Pvt. Ltd, India
Peceol (Glycerol Monooleate)	Central Drug House Pvt. Ltd, India

Methanol AR	S.D.Fine-Chem Ltd, India
Sodium Bicarbonate	Central Drug House Pvt. Ltd, India
Sodium Chloride	Central Drug House Pvt. Ltd, India
Sodium Hydroxide	Central Drug House Pvt. Ltd, India
Calcium Chloride Dihydrate	Central Drug House Pvt. Ltd, India
Benzylkonium Chloride	Finar Chemicals Pvt. Ltd, India

4.2 Instrument used:

INSTRUMENTS	**VENDOR'S NAME**
Digital balance	Citiweigh -Tejas Exports, India
Electronic Weighing Balance	Balance Shimadzu Corporation Ltd. Japan
Mechanical Stirrer	Remi Motors Ltd. India
Vortex shaker	Remi Motors Ltd. India
Ultraviolet spectrophotometer	Shimdzu UV 1800 Corporation, Japan
Refrigerated micro centrifuge	Rajendra Electrical Industries Ltd, India
Humidity control oven	Nova Instruments Pvt. Ltd, India
Ultrasonicator	Trans-O-Sonic D-Compact, India
pH meter	Analab Scientific Instruments, India.
Brookfield viscometer	Brookfield Engineering Laboratories, Usa
Fourier Transformed Infra Red spectrophotometer	Spectrum- GX, Perkin Elmer, USA

4.3 IDENTIFICATION OF NATAMYCIN :

4.3.1 Melting Point Determination

Melting point is the temperature at which the pure liquid and solid exist in the equilibrium. In the practice it is taken as equilibrium mixture at an external pressure of 1 atmosphere; this is sometime known as normal melting point. The thiel's tube method of melting point determination in liquid paraffin was used in the present study.

4.3.2 Determination by UV spectroscopy

20 mg of Natamycin was dissolved in 0.1% glacial acetic acid and methanol mixture and volume was made up to 100 ml. 3 ml of resulting solution was taken and diluted it up to 100 ml with methanol. Now, the resulting solution was examined using UV spectrophotometer between 220 nm and 400 nm.

4.4 ESTIMATION OF NATAMYCIN

4.4.1 Standard curve of Natamycin in Simulated Tear Fluid (STF) (pH 7.4)

Preparation of stock solution

10 mg of Natamycin was accurately weighed and transferred in 100 ml volumetric flask. It was dissolved in 0.1 % glacial acetic acid and methanol mixture and volume was made up to the mark with Methanol to get 100 µg/ml solution.

Preparation of standard curve in STF (pH 7.4)

1st dilution: 10 ml of the stock solutions were pipetted out into a 100 ml volumetric flask and the volume was maintained up to the mark with simulated tear fluid (pH 7.4).
2nd dilution: From above dilution solution 1,2,…10 ml solution were transferred to 10 ml volumetric flask and diluted with STF to give drug concentration of 1,2,..,10 µg/ml respectively. Absorbance of each solution was measured at λ_{max} 304 nm using UV spectrophotometer.

4.4.2 Calibration curve of Natamycin for determination of drug content in microemulsion :

A standard stock solution of Natamycin was prepared in 0.1% glacial acetic acid and methanol mixture by dissolving 50 mg natamycin in 250 ml methanol, suitable dilutions were made from the standard solution to get concentrations in the range of 1-10µg/ml. The absorption maxima (λmax) was determined by scanning 5 µg/ml solution against the reagent blank on UV-visible spectrophotometer and the absorbance maxima was found out at 303 nm. The absorption of all the prepared solutions was then measured at the absorption maxima, 303 nm, against the reagent blank. The readings were recorded in triplicate and the experiment was repeated on 3 consecutive days using freshly prepared stock solutions each time. Mean values (n=3) along with the standard deviation are recorded and the regressed calibration curve was developed.

4.5 Solubility studies and selection of surfactant and oil component [40]

The solubility of Natamycin in various oils, surfactants, and co-surfactants was determined visually and then they were quantified.

Each of selected vehicles (1 ml) was added to each eppendorp tube containing an excess of natamycin. After sealing, the mixture was heated at 40 ºC in water-bath to facilitate the solubilization and mixed using a vortex mixer.

Mixtures were shaken on shaker bath at 30 ºC for 48 h. After reaching equilibrium, each tube was centrifuged at 12,000 rpm for 10 min, then 0.5 ml supernatant was taken with micropipette, and the content of natamycin was quantified by UV-Visible spectrophotometer 303 nm after dilution with methanol.

4.6 EVALUATION

4.6.1 pH MEASUREMENTS

The pH for each formulation was measured for using a pH meter, which was calibratedn before use with buffered solutions of pH 4.0 and pH 7.0.

4.6.2 VISCOSITY

The rheological property of the microemulsion formulations was evaluated using small sample adaptor and spindle no. 18 of the Brookfield Viscometer (LVDV-I Prime model). The viscosity was measured at 0.5 rpm speed. Evaluations were conducted in triplicate.

4.6.3 PARTICLE SIZE DISTRIBUTION

The particle size distribution of the oil droplets in the microemulsion was analyzed using a Mastersizer without dilution at 25 ºC.

4.6.4 SOLUBILITY OF NATAMYCIN IN O/W MICROEMULSION [40]

An excess amount of Natamycin was introduced to 1 ml of microemulsion in eppendorp tube. After sealing, the mixture was heated at 40ºC in water-bath to facilitate the solubilization and mixed using a vortex mixer. Mixtures were shaken with shaker bath at 25ºC for 48 h. After reaching equilibrium, each tube was centrifuged at12,000 rpm for 10 min , then 0.5 ml supernatant was taken and the content of Natamycin was quantified by UV-Visible spectrophotometer at 303 nm after dilution with methanol.

4.6.5 DRUG CONTENT

Drug content in formulation was determined by dissolving 100µl quantity of formulation in 10 ml of 0.1 % glacial acetic acid and methanol mixture . The solution was then filtered through 0.45µm membrane filter and analyzed for Natamycin content by UV-visible spectrophotometer at 303 nm.

4.6.6 Thermodynamic stability of microemulsions [41]

Thermodynamic stability was examined for both pre-microemulsion formulations (i.e., natamycin-free formulations) and microemulsions containing natamycin through the following procedure:

(i) Heating-cooling cycle –

Six cycles were carried out between refrigerator temperature (4°C) and 45°C with storage at each temperature of no less than 48 h. The formulations that were stable at these temperatures were subjected to a centrifugation test.

(ii) Centrifugation test –

Passing formulations were centrifuged at 3,500 rpm for 30 min. Those formulations that had no phase separation were used in a freeze-thaw stress test.

(iii) Freeze-thaw cycle -

Three freeze-thaw cycles were carried out between –21°C and 25°C with storage of formulations at each temperature for no less than 48 h .

4.6.7 In vitro permeation study [42]

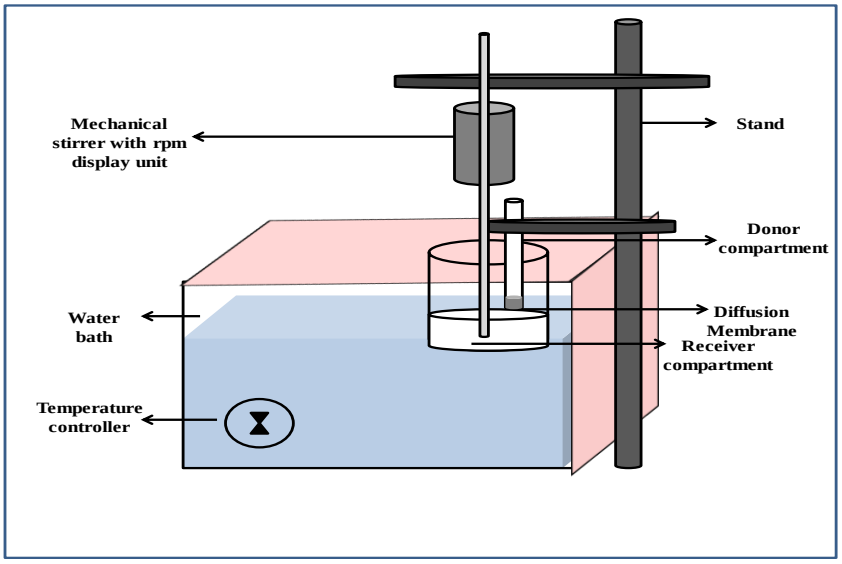


Figure 4.1 : Diffusion Assembly Set up

To determine the in vitro permeation of Natamycin, assembly was set as shown in figure 4.1 , 100 ml of artificial tear fluid was placed in a beaker and mounted vertically in a

water bath at 34±0.1 ^{o}C. 1ml of formulation was added to donor compartment tied with the cellophane membrane at lower end. The temperature and stirring rate were remained at 34 ºC and 75 rpm, respectively. Aliquots of 5 ml were withdrawn from the release medium and replaced by an equal volume with STF at each sampling time.

The amount of Natamycin was determined by UV-visible spectrophotometer. Cumulative amount of drug (*Qn*, µg/cm2) in the receiver compartment was plotted as a function of time (*t*, min), and the cumulative amount of Natamycin permeated through membrane from Nata drop Eye drop solution and formulated microemulsion was determined based on the following equation .

$$Qn = (Cn * V_0 + \sum_{i=1}^{n-1} Ci * Vi) / S$$

Where, *Cn* stands for the drug concentration of the receiver medium at each sampling time, *Ci* for the drug concentration of the *i* th sample, and V_0 and *V* stand for the volumes of the receiver solution and the sample, respectively, *S* for the effective diffusion area.

4.7 IN SITU GELLING SYSTEM:

4.7.1 Formulation of in situ gel [29]

The carbopol 940 solutions were prepared by dispersing the required amount in 75ml distilled, deionized water with continuous stirring until it was completely dissolved. Similarly HPMC K4M solutions were prepared by dispersing the required amount of distilled, deionized water in the with continuous stirring until completely dissolved. Both of them were mixed then to obtain solution. The pH was adjusted to 6.3 using 0.5 M sodium hydroxide. Benzalkonium chloride (BKC) was then added to the above solution. Distilled, deionizd water was then added to make the volume up to 100 ml.

4.7.2 Evaluation:

3.7.2.1 Gelling capacity [29]:

The gelling capacity was determined by placing a drop of the system in a vial containing 2 ml of freshly prepared artificial tear fluid and equilibrated at 37 ºC and visually assessing the gel formation and noting the time for gelation and the time taken for the gel formed to dissolve.

The composition of artificial tear fluid used was sodium chloride 0.670 g, sodium bicarbonate 0.200 g, calcium chloride $2H_2O$ 0.008 g, purified water q.s. 100.0 g. The viscosity was measured using a Brookfield Synchrolectric viscometer (RVT model) in the small volume adaptor. The viscosity measured at 20 rpm was used for purposes of comparative evaluation.

Table 4.1: Gelling capacity

-	No Gelation
+	Gels after a few min., dissolved rapidly
+ +	Gelation immediate, remains for nearly 6 hours
+++	Gelation immediate, remains for nearly 10 hours
++++	Gelation immediate, remains for more than 12 hours

3.7.2.2 Rheological Studies [29]:

The developed formulation was poured into the small sample adaptor of the Brookfield synchrolectric viscometer and the angular velocity was increased gradually from 0.5 to 100 rpm. The hierarchy of the angular velocity was reversed. The average of two readings was used to calculate the viscosity. The developed system was poured into an ointment jar, and the pH of the formulation was increased to 7.4 by adding 0.5 N sodium hydroxide solution. The rheology of the resultant gel was studied using the S 92.

4.8 FORMULATION OF MICROEMULSION IN SITU GELLING SYSTEM

In previous sections, suitable compositions of microemulsion and in situ gelling system were individually screened for the desired properties and they were used for further optimization of ME based in situ gelling system.

Step 1- Appropriate amount of oil , surfactant and co surfactant was mingled together in accordance to ME domain, and equilibrated with gentle vortex shaking to get the initial concentrate. Then appropriate natamycin was dissolved in the initial concentrate under ultra-sonication [43].

Step 2- carbopol 940 and HHPMC K4M was dispersed in sufficient deionized water separetely until it completely dissolved using magnetic stirrer and both of them were mixed to obtain solution.

Step 3- Natamycin loaded microemulsion slowly add in carbopol 940 and HPMC K4M with stirring.

4.9 Evaluation :

4.9.1 pH measurements

The pH was measured for each formulation using a pH meter, which was calibrated before use with buffered solutions of pH 4.0 and 7.0.

4.9.2 Rheological studies [42]

The viscosity of the optimized formulation was determined at different angular velocities at 34±1 ° C using small sample adaptor of the Brookfield Viscometer (LV model). A typical run involved changing the angular velocity from 0.5 to 100rpm at a controlled ramp speed. After 6 s at 0.5 rpm, the velocity was successively increased to 100 rpm, with a similar period at each speed. The angular velocity was then decreased (100-0.5 rpm) for a similar period of 6 s. The average of two readings was used to calculate the viscosity. Evaluations were conducted in triplicate.

4.9.3 In vitro release studies [42]:

To determine the in vitro release of Natamycin, assembly was set as shown in figure 3.1, 100 ml of artificial tear fluid was placed in a beaker and mounted vertically in a water bath at 34±0.1 ºC . 1ml of formulation was added to donor compartment tied with the cellophane membrane at lower end. The temperature and stir rate were remained at 34 ºC and 75 rpm, respectively. Aliquots of 5 ml were withdrawn from the release medium and replaced by an equal volume with STF at each sampling time. The amount of Natamycin release was determined by UV-visible spectrophotometer and cumulative amount release, % was plotted as a function of time (*t*, min).

Chapter 5
Experimental Work

IDENTIFICATION OF NATAMYCIN:

5.1.1 Melting Point Determination

Description:

Melting point is the temperature at which the pure liquid and solid exist in the equilibrium. In the practice it is taken as equilibrium mixture at an external pressure of 1 atmosphere; this is sometime known as normal melting point. The thiel's tube method of melting point determination in liquid paraffin was used in the present study. Melting -point was found to be 279 ˚C.

Table 5.1 : Compare melting point of Natamycin

Actual melting point	280˚C
Observed melting point	277- 279 ˚C

Result:

The melting point of Nataycin was found to be 277- 279 ˚C.

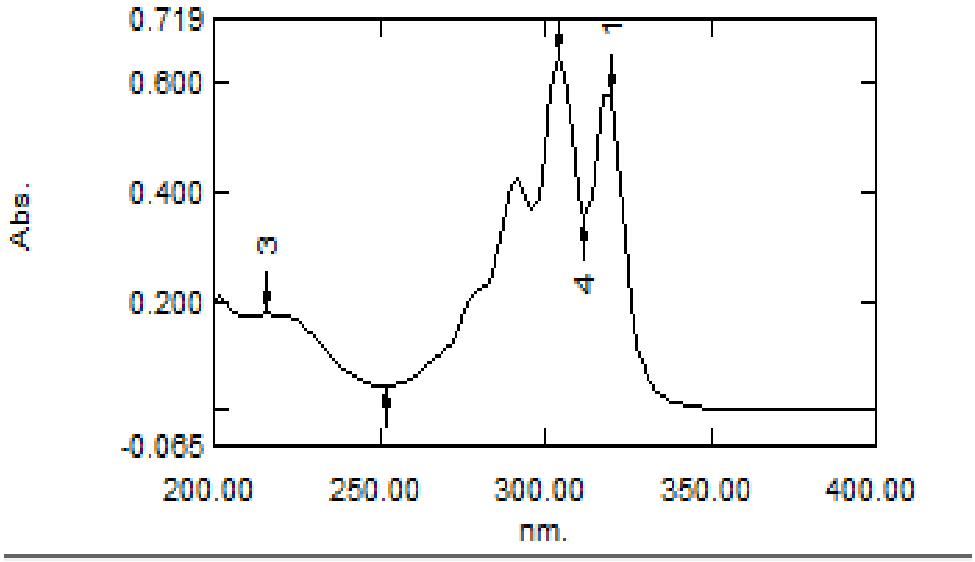
Conclusion:

The melting point determined is within the range of standard value, hence, it is concluded that the drug sample having intimate physical property as standard drug.

5.1.2 Determination by UV spectroscopy.

20 mg of Natamycin was dissolved in 0.1% glacial acetic acid and methanol mixture and volume was made up to 100 ml. 3 ml of resulting solution was taken and diluted it up to 100 ml with methanol. Now, the resulting solution was examined using UV spectrophotometer between 220 nm and 400 nm.

Figure 5.1: UV spectra of Natamycin in Methanol + 0.1 % Glacial acetic acid



Result :

The above UV spectra of Natamycin show the λmax at 302 nm,.

Conclusion:

The above UV spectra of Natamycin shows the λmax at 302 nm,.which was similar to the reported standard value 303 nm.

5.2 ESTIMATION OF NATAMYCIN

5.2.1 Standard Curve of Natamycin in Methanol :

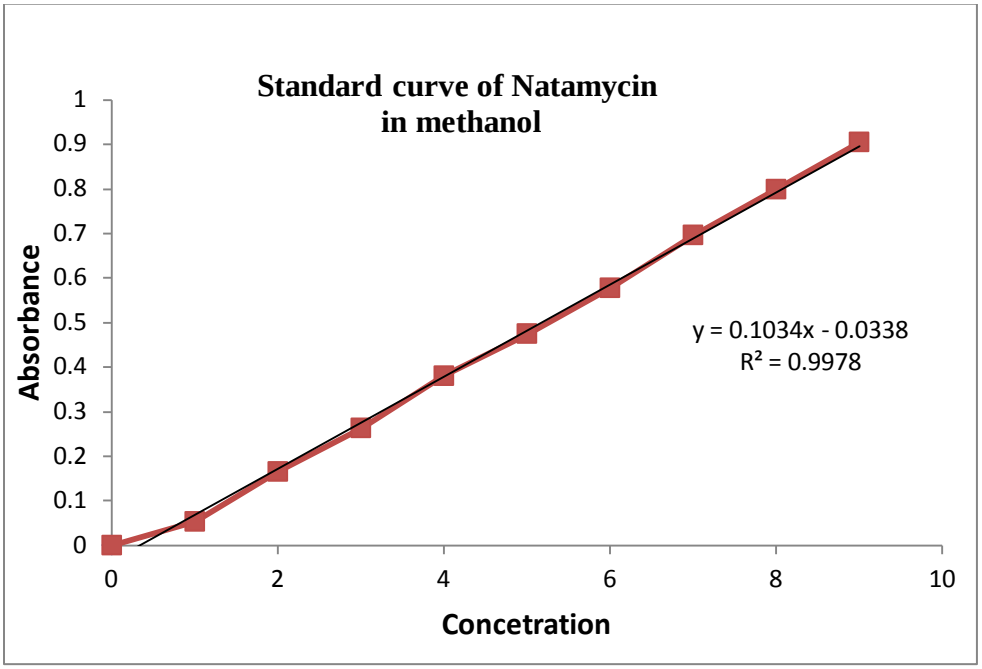


Figure 5.2: Standard curve of Natamycin in Methanol

Regression Analysis

Table 5.3: Regression Analysis for : Standard curve of Natamycin in methanol

Regression parameter	Value
Correlation coefficient	0.9978
Slope	0.1034
Intercept	0.0338

5.2.2 Standard curve of natamycin in STF

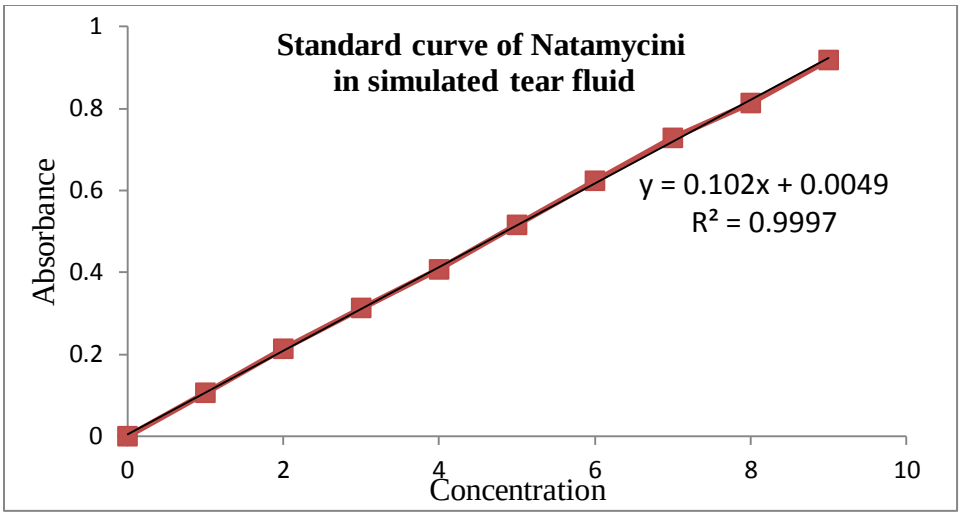


Figure 5.3: Standard Curve of Natamycin in STF

Regression Analysis

Table 5.5: Regression Analysis for Standard Curve of Natamycin in STF

Regression parameter	Value
Correlation coefficient	0.9997
Slope	0.102
Intercept	0.0049

5.3 Selection of oil and surfactant by solubility:

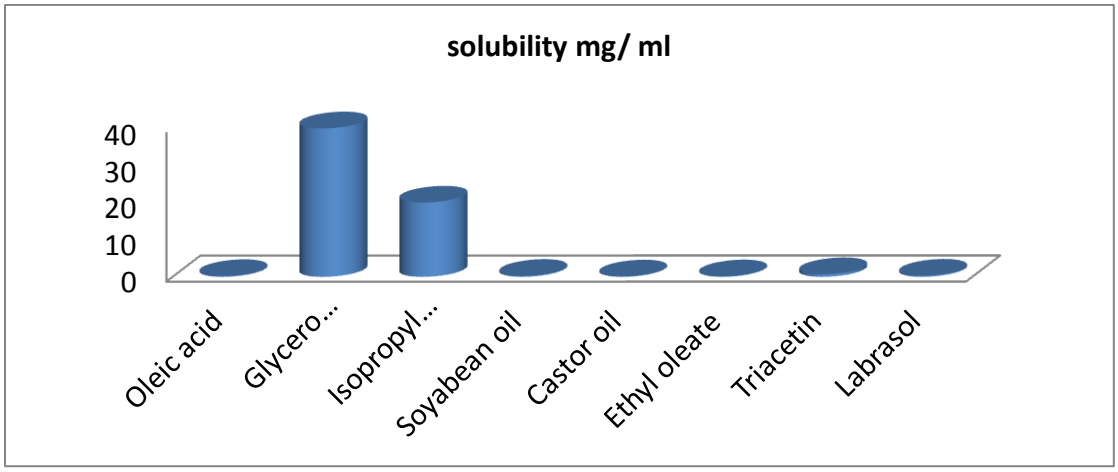


Figure 5.4: Solubility of natamycin in various Oil

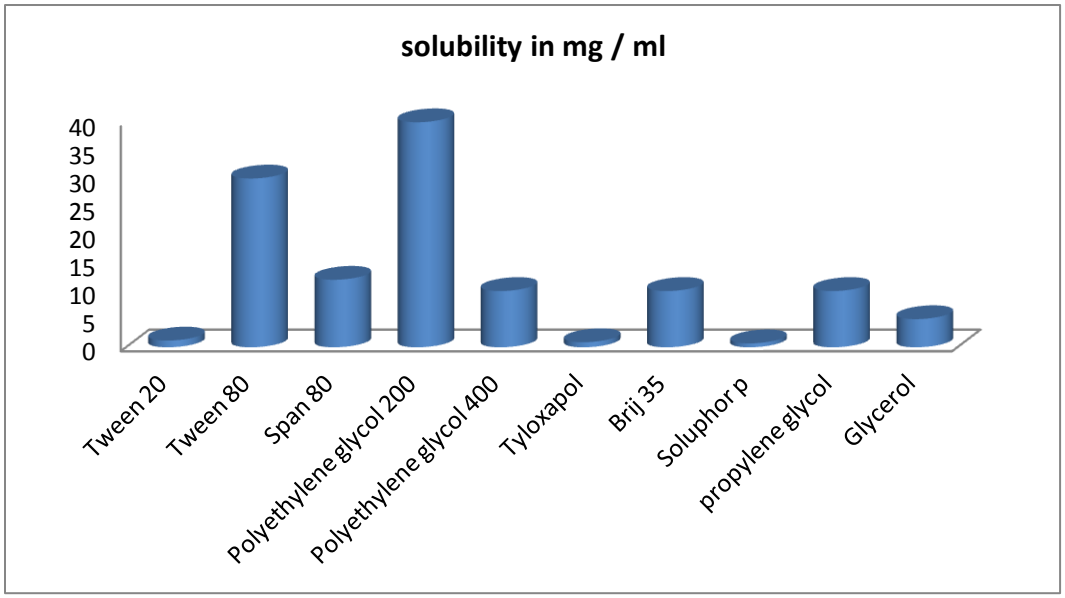


Figure 5.5: Solubility of natamycin in various Surfactant

Conclusion :

From the above result it can be concluded that Natamycin has highest solubility in oils such as like Glycero Monooleate (GM) and IsoPropyl Myristate (IPM) , and surfactants like Tween 80 and PEG 200. IPM was selected as oil because Glycero Monooleate remain as solid form below 38 ºC so that it makes instable microemulsion, and Tween 80 and PEG 200 selected as surfactant and cosurfactant respectively..

5.4 Construction of pseudo-ternary phase diagrams:

In order to find out the concentration range of components for the existence range of microemulsion, pseudo-ternary phase diagrams were constructed using water titration method at ambient temperature (25 ºC). The phase diagrams were prepared with the 1:1, 2:1, 1:2, 1:3, 1:4, 1:5, 1:6, and 1:7 ratios of Tween 80 to PEG 200 respectively. For each phase diagram at specific surfactant/cosurfactant w ratio, the ratios of Smix and oil were varied as 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8 and 1:9. The mixtures of oil, surfactant and cosurfactant at certain ratios were diluted with water dropwise, under moderate magnetic stirring. After being equilibrated, the mixtures were assessed visually and determined as being microemulsions, crude emulsions or gels.

Figure 5.6: Ternary phase diagram of 1:1 Surafactant : cosurfactant and Oil

S : cos (1:1)

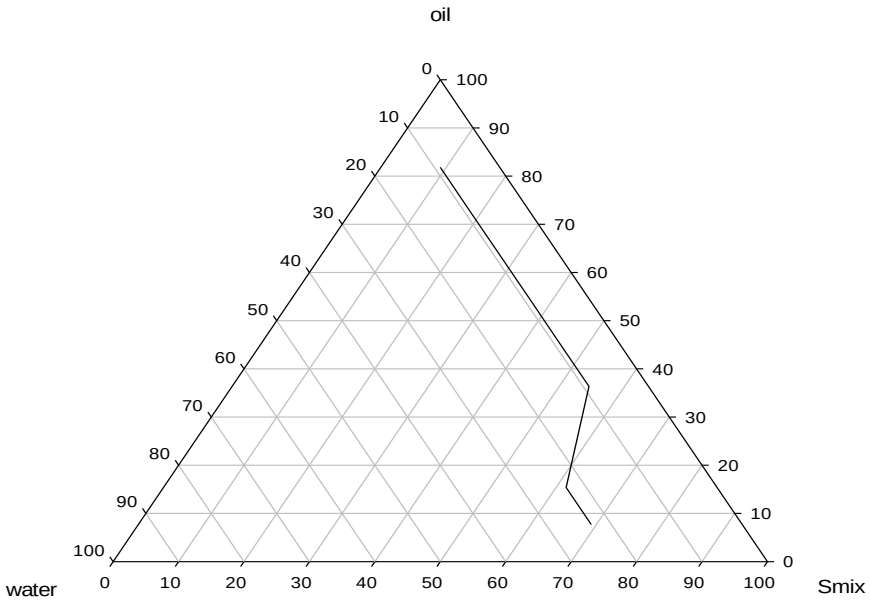


Figure 5.7: Ternary phase diagram of 2:1 Surafactant : cosurfactant and Oil

S : cos (2:1)

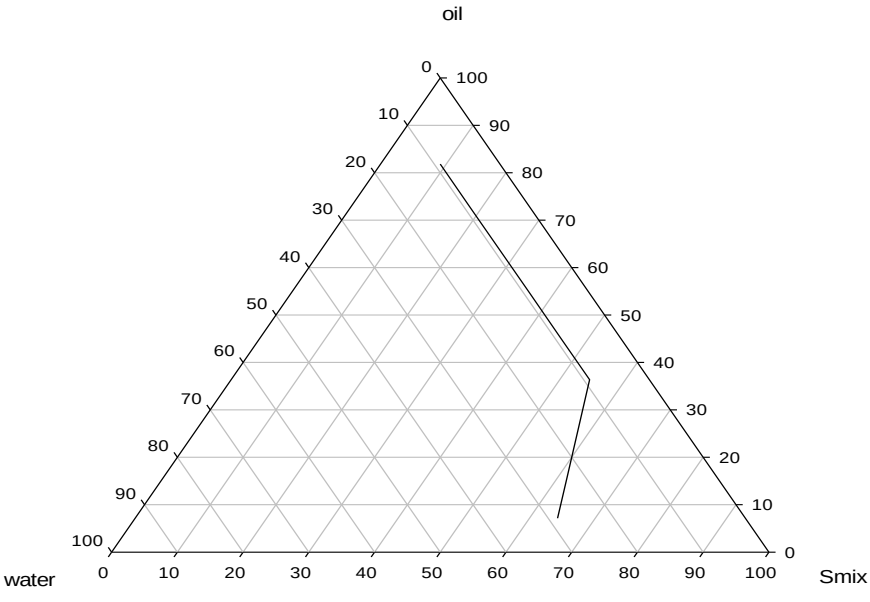


Figure 5.8: Ternary phase diagram of 1:2 Surafactant : cosurfactant and Oil

S:COS (1:2)

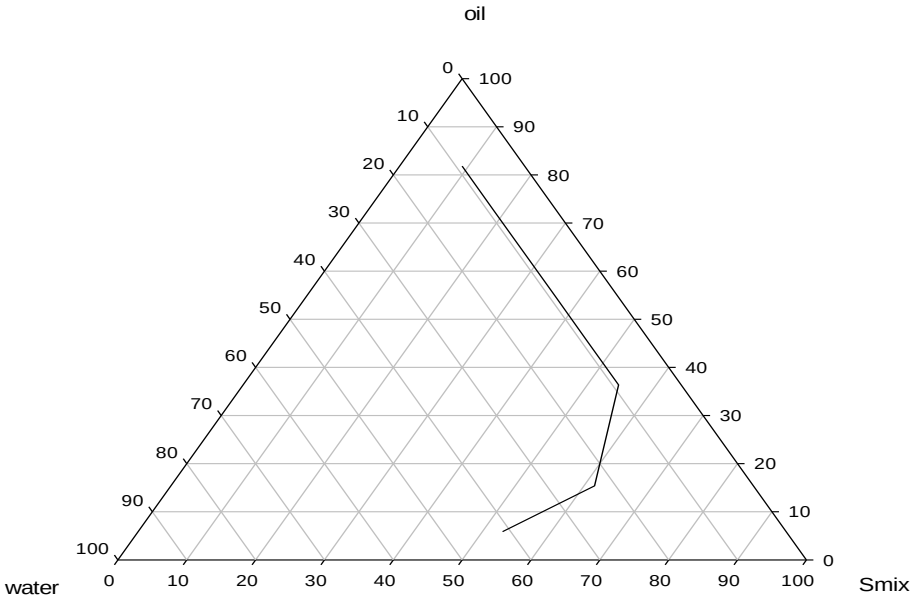


Figure 5.9: Ternary phase diagram of 1:3 Surafactant : cosurfactant and Oil

S:COS (1:3)

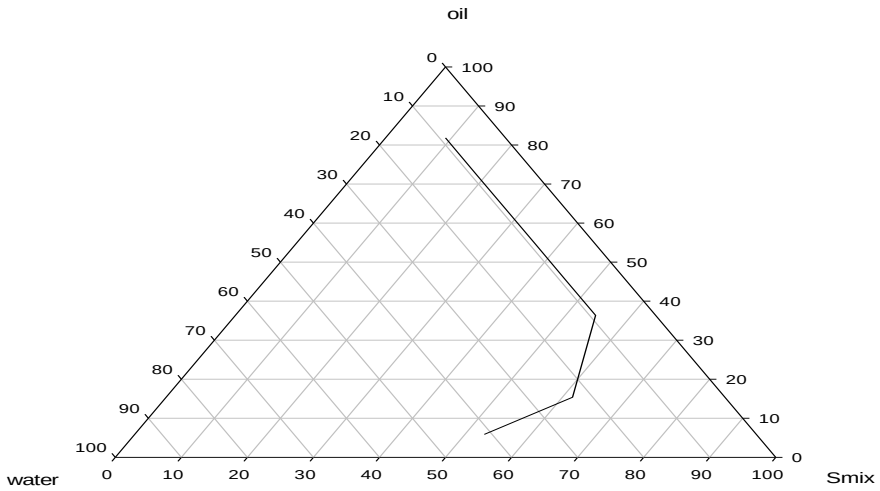


Figure 5.10: Ternary phase diagram of 1:4 Surafactant : cosurfactant and Oil

S:COS (1:4)

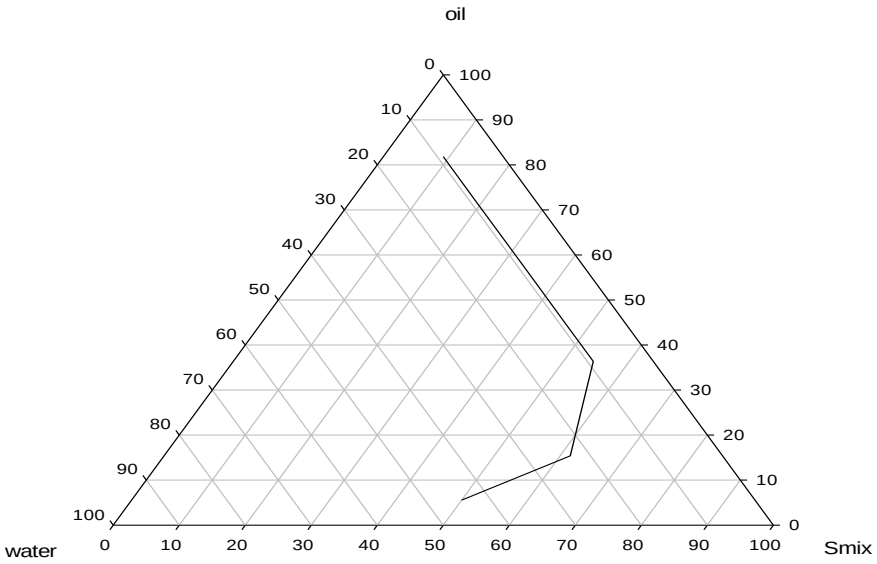


Figure 5.11: Ternary phase diagram of 1:5 Surafactant : cosurfactant and Oil

S:COS (1:5)

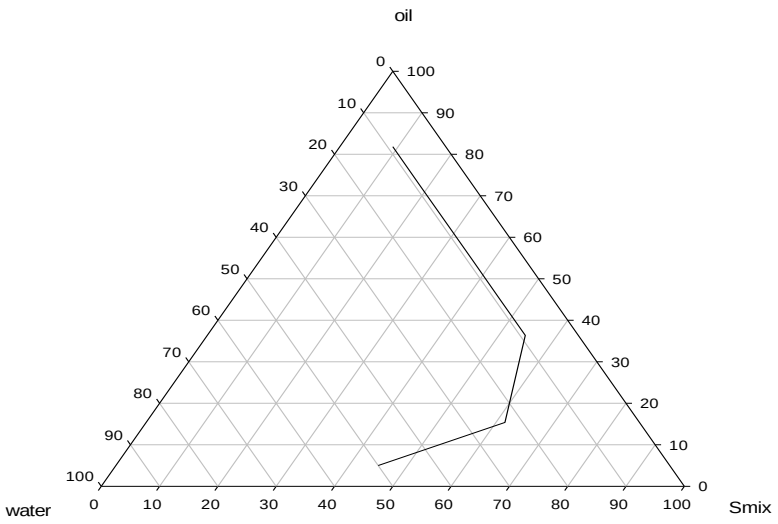


Figure 5.12: Ternary phase diagram of 1:6 Surafactant : cosurfactant and Oil

S:COS (1:6)

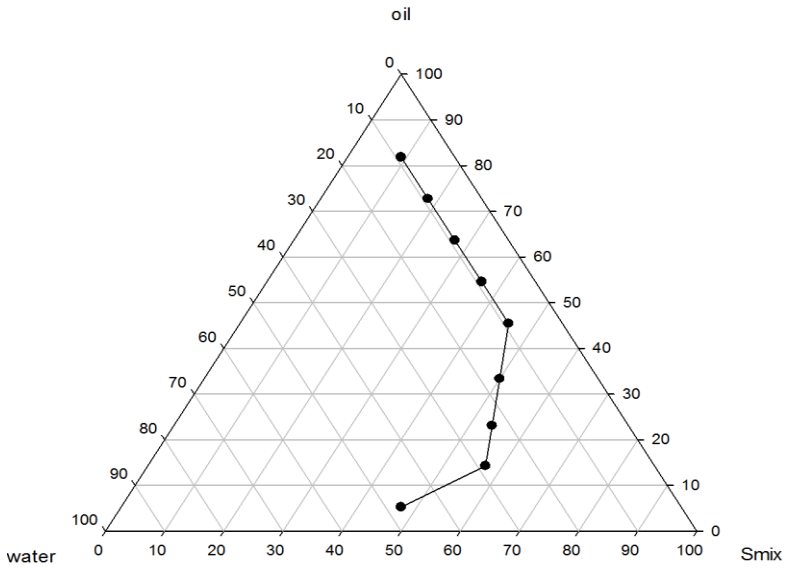
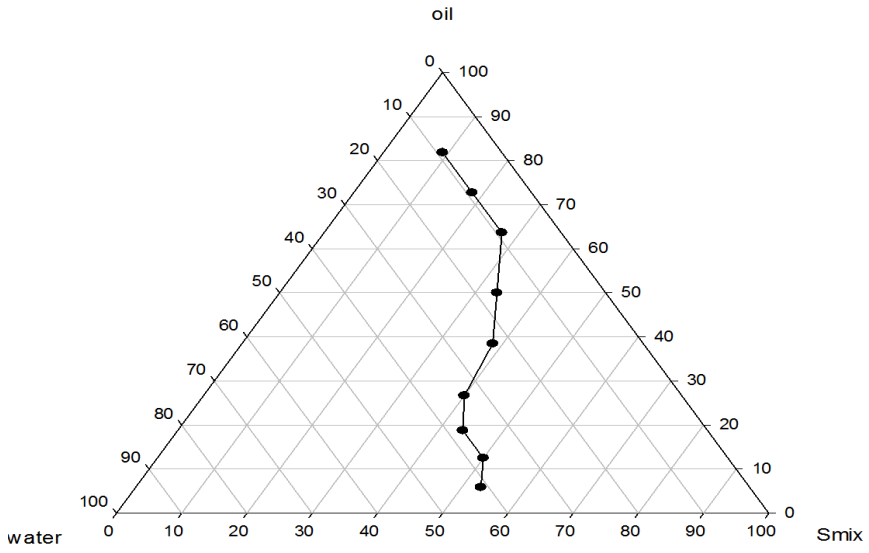


Figure 5.13: Ternary phase diagram of 1:7 Surafactant : cosurfactant and Oil

S:COS (1:7)



5.4.1 EVALUATION :

5.4.1.1 Percentage microemulsion existence area

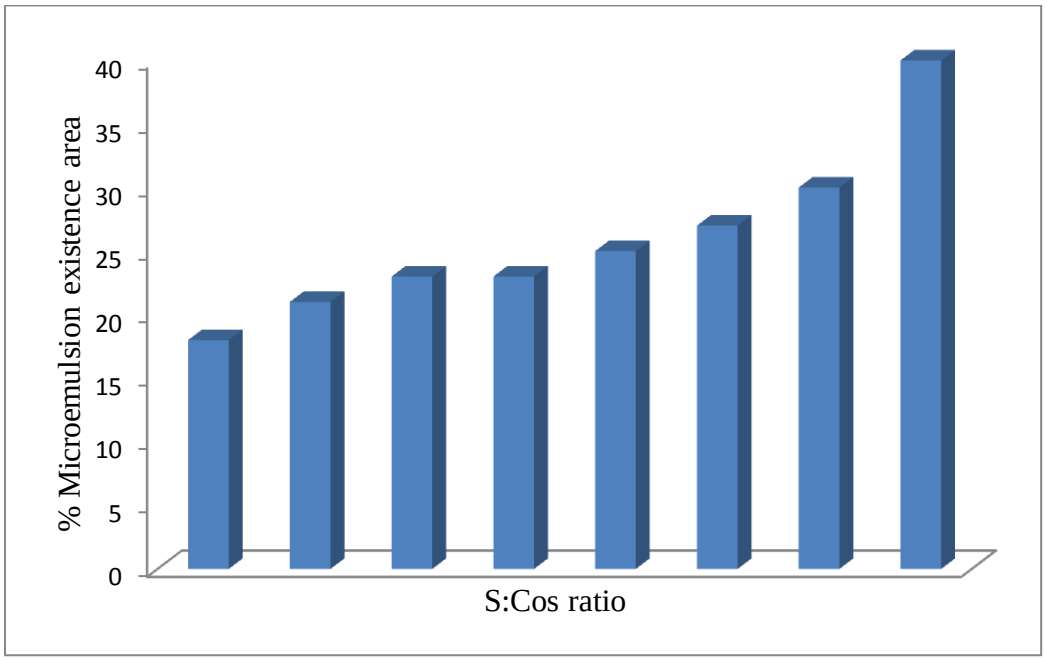
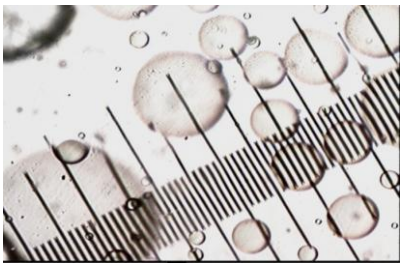


Figure 5.14: Percentage microemulsion existence area

5.4.1.2 Photograph of microemulsion under microscope (10X)

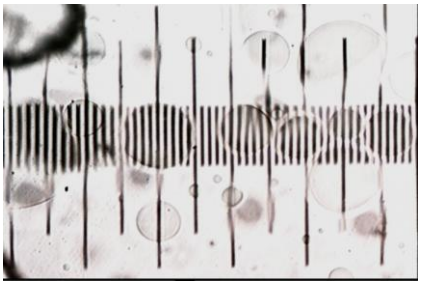


Tween 80 :PEG (1:1)

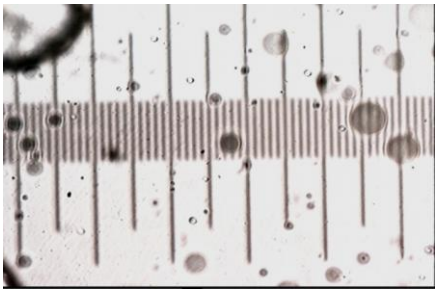
Tween 80:PEG (1:2)

Tween 80 :PEG (2:1)

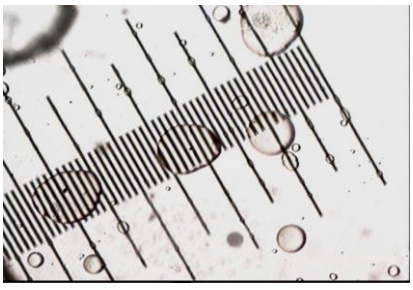
Tween 80 :PEG (1:3)



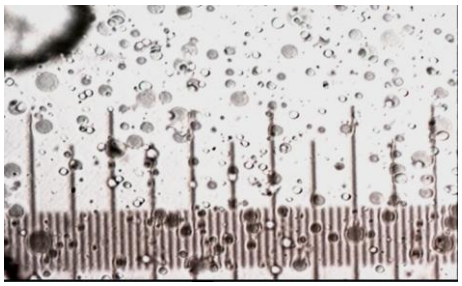
Tween 80 :PEG (1:4)



Tween 80:PEG (1:5)



Tween 80 :PEG (1:6)



Tween 80:PEG (1:7)

Discussion:

From all ternary diagram it can be concluded that as concentration of co-surfactant increases , the area of microemulsion increase , the globule size decrease with increase in stability .

As Tween 80:PEG 200 (1:7) ratio show lowest globule size and good stability , it was fixed for the formulation of microemulsion. Further the Smix to oil ratio was changed and titrated with water .

S:COS (1:7)

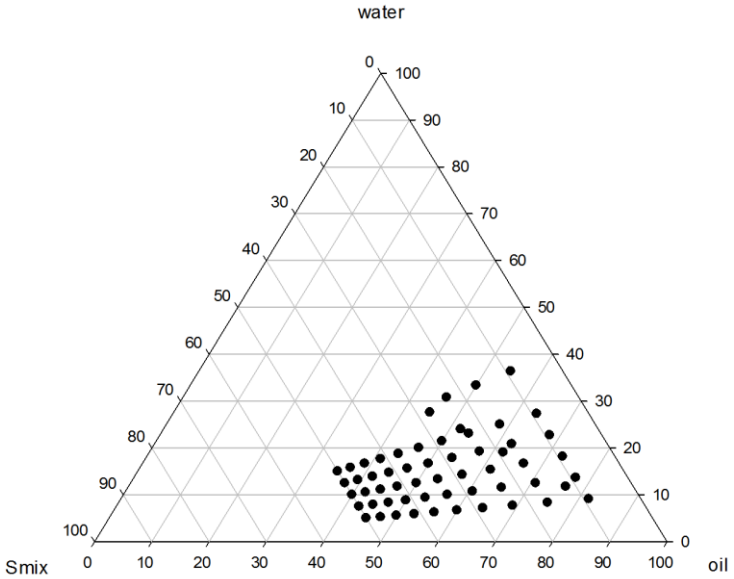
Smix : Oil (9:1)						
Smix	oil	water	total	% Smix	% oil	% water
450	50	50	550	81.82	9.09	9.09
450	50	100	600	75.00	8.33	16.67
450	50	150	650	69.23	7.69	23.08
450	50	200	700	64.29	7.14	28.57
450	50	250	750	60.00	6.67	33.33
450	50	300	800	56.25	6.25	37.50
450	50	350	850	52.94	5.88	41.18
450	50	400	900	50.00	5.56	44.44
450	50	450	950	47.37	5.26	47.37
450	50	500	1000	45.00	5.00	50.00
Smix : Oil (8.5:1.5)						
425	75	50	550	77.27	13.64	9.09
425	75	100	600	70.83	12.50	16.67
425	75	150	650	65.38	11.54	23.08
425	75	200	700	60.71	10.71	28.57
425	75	250	750	56.67	10.00	33.33
425	75	300	800	53.13	9.38	37.50
425	75	350	850	50.00	8.82	41.18
425	75	400	900	47.22	8.33	44.44
425	75	450	950	44.74	7.89	47.37
425	75	500	1000	42.5	7.50	50.00
Smix : Oil (8:2 Smix : Oil (8:2)						

400	100	50	550	72.73	18.18	9.09
400	100	100	600	66.67	16.67	16.67
400	100	150	650	61.54	15.38	23.08
400	100	200	700	57.14	14.29	28.57
400	100	250	750	53.33	13.33	33.33
400	100	300	800	50.00	12.50	37.50
400	100	350	850	47.06	11.76	41.18
400	100	400	900	44.44	11.11	44.44
400	100	450	950	42.11	10.53	47.37
400	100	500	1000	40	10	50
Smix : Oil (7.5:2.5)						
375	125	50	550	68.18	22.73	9.09
375	125	100	600	62.50	20.83	16.67
375	125	150	650	57.69	19.23	23.08
375	125	200	700	53.57	17.86	28.57
375	125	250	750	50.00	16.67	33.33
375	125	300	800	46.88	15.63	37.50
375	125	350	850	44.12	14.71	41.18
375	125	400	900	41.67	13.89	44.44
375	125	450	950	39.47	13.16	47.37
375	125	500	1000	37.5	12.5	50
Smix : Oil (7:3)						
350	150	50	550	63.64	27.27	9.09
350	150	100	600	58.33	25.00	16.67
350	150	150	650	53.85	23.08	23.08
350	150	200	700	50.00	21.43	28.57
350	150	250	750	46.67	20.00	33.33
350	150	300	800	43.75	18.75	37.50
350	150	350	850	41.18	17.65	41.18
350	150	400	900	38.89	16.67	44.44
350	150	450	950	36.84	15.79	47.37
350	150	500	1000	35	15	50
Smix : Oil (6.5:3.5)						
325	175	50	550	59.09	31.82	9.09
325	175	100	600	54.17	29.17	16.67
325	175	150	650	50.00	26.92	23.08
325	175	200	700	46.43	25.00	28.57
325	175	250	750	43.33	23.33	33.33

325	175	300	800	40.63	21.88	37.50
325	175	350	850	38.24	20.59	41.18
325	175	400	900	36.11	19.44	44.44
325	175	450	950	34.21	18.42	47.37
Smix : Oil (6:4)						
300	200	50	550	54.55	36.36	9.09
300	200	100	600	50.00	33.33	16.67
300	200	150	650	46.15	30.77	23.08
300	200	200	700	42.86	28.57	28.57
300	200	250	750	40.00	26.67	33.33
300	200	300	800	37.50	25.00	37.50
300	200	350	850	35.29	23.53	41.18
300	200	400	900	33.33	22.22	44.44
300	200	450	950	31.58	21.05	47.37
300	50	500	850	35.29	5.88	58.82

Note : Dark area show the microemulsion existence

Figure 5.15: Ternary Phase Digram All Point Where Microemulsion Existence:



Conclusion:

From above ternary phase diagram . there was decided to formulate microemulsion range of Smix 50 to 70 % , range of oil 10 to 20 % and range of water 10 to 40 % .

5.5 Microemulsion preparation:

Table 5.16: Compositon of Natamycin (1 %) loaded Microemulsion

Ingredient	A	B	C	D	E	F	G	H	I
%Smix	50	50	50	60	60	60	70	70	70
%IPM	10	15	20	10	15	20	10	15	20
%Water	40	35	30	30	25	20	20	15	10

5.5.1 Evaluation :

Figure 5.16: Comparison of particle size of various microemulsion batches :

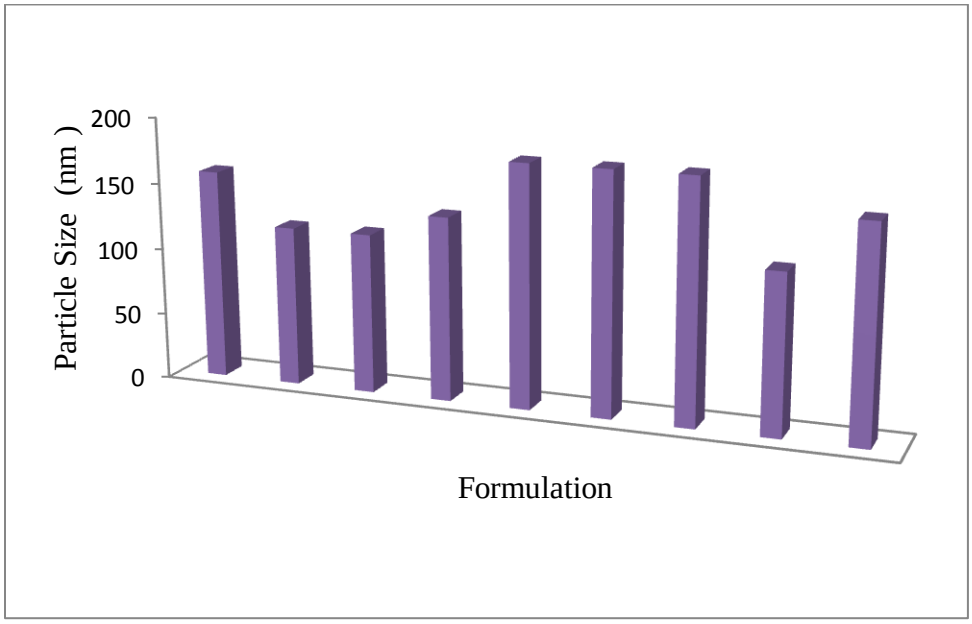
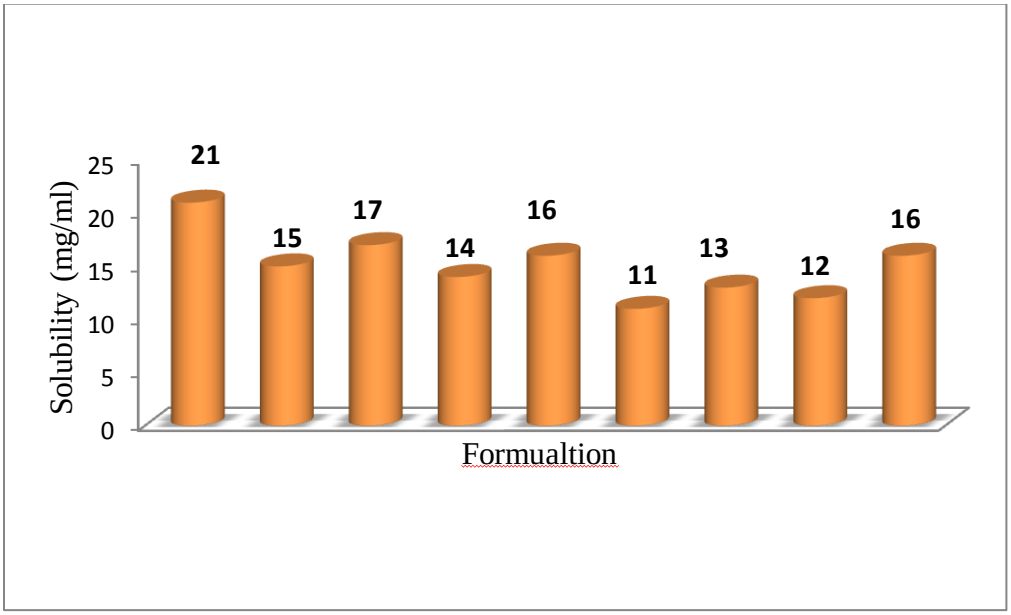


Figure 5.17: Comparison of solubility of various microemulsion batches



5.6 IN SITU GEL

Table 5.20: Preparation of carbopol 940 In-situ ophthalmic gel

(Optimization of carbopol 940 concentartion)

Ingredient	C1	C2	C3	C4	C5	C6
Natamycin (% w/v)	1	1	1	1	1	1
Carbopol 940 (%w/v)	0.1	0.2	0.3	0.4	0.5	0.6
Sodium Chloride (%w/v)	0.9	0.9	0.9	0.9	0.9	0.9
Benzylkonium chloride(BAK) (%w/v)	0.02	0.02	0.02	0.02	0.02	0.02
De ionized water	Up to 100 ml	Up to 100 ml	Up to 100 ml	Up to 100 ml	Up to 100 ml	Up to 100 ml

Result:

Parameter	C1	C2	C3	C4	C5	C6
Gelling Capacity	+	+	+++	+++	+++	+++
Instillation (Dropping capacity)	Easily	Easily	Easily	Easily	Easily	Moderate

Conclusion:

From above result we concluded that Batch C1 and C2 do not have good gelling capacity and gel gets dissolved within an hour due to presence of low amount of gelling agent. Batches C3 to C5 have good gelling capacity and remained as gel up to 12 hour.

Batch C3 shows good gelling capacity in low concentration and hence was selected for further optimization of viscosity studies.

Due to less viscosity of prepared batches C1 to C6 there was need to add viscosity enhancers like methyl cellulose and HPMC K4M to improve rheological behaviour of formulation.

RHEOLOGY STUDY:

Figure 5.19: Rheological profile of in situ gelling system:

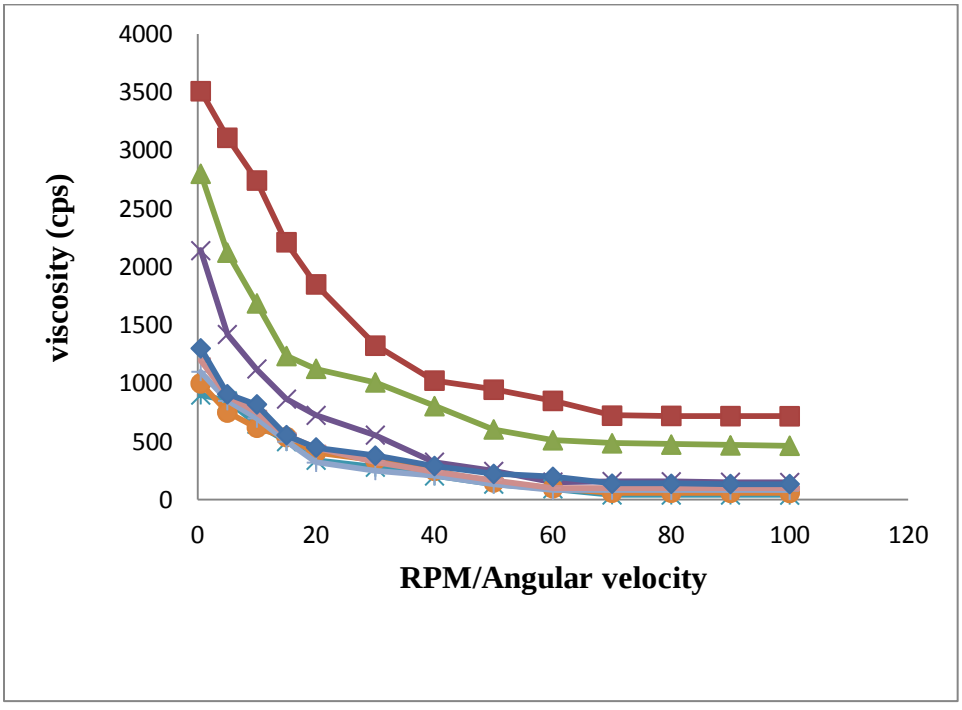
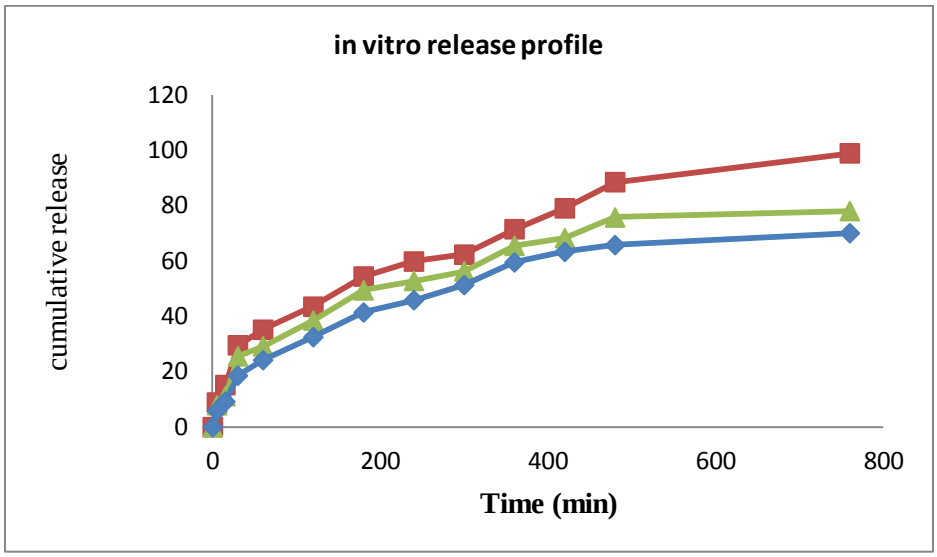


Figure 5.20: Comparison of in vitro release of of in situ gelling system



Discussion:

From above result we concluded that batch C10 showed good gelling and dropping capacity and drug release up to 99 % within 12 hours which was more than other batches hence batch C10 was the best batch for preparation of microemulsion in situ gel of Natamycin.

Chapter 6
Summary

SUMMARY

Solubility of drug in different oil and surfactant was determined. Natamycin showed higher solubility in IPM , Tween 80 and PEG 200. So they selected as oil, surfactant and co – surfactant respectively.

To select prepare microemulsion region, different properties of oil, Smix and water were tried. Initially, surfactant and cosurfactant were mixed together in oil in ration of (1:1, 1:2,1:3, 1:4, 1:5, 1:6 and 1:7) and finally mixed with oil in ration of 9:1, 8.5:1.5, 8:2, 7.5:2.5, 7:3, 6.5:3.5, 6:4 respectively. Water was added dropwise, with moderate stirring and psuedoternary phase diagram were prepared.

Tween80:PEG (1:7) ratio show lowest globule size and good stability , it was fixed to the formulation of microemulsion. Further the Smix to oil ratio was changed and titrate with water.

Based on final ternary phase diagram , Various microemulsion Batches were prepared .

Batch A has low particle size ,high drug solubility capacity and high in vitro permeation of Natamycin as compared to all other bathces and even high in vitro permeation of Natamycin as compared to marketed formulation , so Batch A is selected as optimized batch .

The in situ gelling system was developed using carbopol 940 from 0.1 to 0.6 concetration and optimized by evaluating gelling capacity, dropping capacity, pH, viscosity.Batch C3 contain 0.3 % carbopol showed good gelling capacity at low concentration so it was selected as best batch and was further optimized for viscosity like methylcellulose and HPMC K4M.

The in situ gelling system was developed using carbopol (0.3 %) with methyl cellulose or HPMC K4M . Batch C10 contain carbopol 0.3 % and HPMC K4M

showed good gelling and dropping capacity and drug release up to 99 % within 12 hours which was more than other batches and hence batch C10 was the consider as best batch for preparation of microemulsion in situ gel of Natamycin**.**

Chapter 7
Future Prospective

FUTURE PROSPECTIVE

The prospects are:

Determination of Ex- vivo permeation.

Determination of In vivo-In vitro correlation of drug release studies.

Determination of pharmacokinetics of drug in vitreous humor after topical administration.

Clinical trials to determine concentration of drug in vitreous humor of human eye.

Comparison of vitreous penetration of drug in rabbit and human eye.

Stability of microemulsion based in situ gelling formulations.

Chapter 8
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